Neurobiopsychologische Analyse des Angstverhaltens im Modell der Ratte: Auswirkung serotonerger Manipulationen

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1. Zusammenfassung

Das Ziel der vorliegenden Arbeit ist die genauere Untersuchung der psychologischen und neurochemischen Zusammenhänge zwischen der Emotion Angst und dem Neurotransmitter Serotonin (5-Hydroxytryptamin, 5HT). Die Literatur bietet diesbezüglich eine Vielzahl von Arbeiten, doch die Funktion von 5HT im Zusammenhang mit Angst ist immer noch nicht vollständig geklärt. Studien, die Angstverhalten anhand von 5HT-Läsionen untersuchen, zeigen inkonsistente Ergebnisse (Griebel 1995, Menard & Treit 1999). Dies könnte unter anderem daran liegen, dass die meisten Studien mit einer generellen Läsion von zentralem 5HT arbeiten, 5HT jedoch durch weit verzweigte Projektionen (Feldman et al. 1997) und verschiedene 5HT-Rezeptorsubtypen (Lesch et al. 2003, Hennig & Netter 2005) Angst unterschiedlich moduliert (Deakin & Graeff 1991, Graeff et al. 1997). Zudem existieren individuelle Unterschiede verschieden ängstlicher Ratten (Schwarting et al. 1998), die sich auf 5HT-Manipulationen auswirken können. Im Rahmen dieser Dissertation wurden drei Studien durchgeführt, die sich mit diesen, bisher meist nicht berücksichtigten, Aspekten beschäftigen und beispielsweise individuelle Unterschiede oder die funktionalen Rolle von 5HT im ventralen Striatum mit einbeziehen. Das Angstverhalten von Ratten wurde nach Manipulation des 5HT-Systems, durch das Toxin 5,7-Dihydroxytryptamin (5,7-DHT), das potentiell neurotoxische 3,4-Methylenedioxymethamphetamin (MDMA) und den $5HT_{2C}$-Antagonisten RS102221 erhoben. Eine Injektion von 5,7-DHT in das ventrale Striatum führte zu einer 5HT-Läsion im anterioren Vorderhirn (Striatum und frontaler Kortex), wobei eine Vorbehandlung mit verschiedenen Wiederaufnahmehemmern notwendig war, um unter anderem das dopaminerge Transmittersystem zu schützen. Studien, die ebenfalls mit gezielten zentralen Injektionen von 5HT-Toxinen arbeiten, vernachlässigen es oft, weitere Hirnareale und Neurotransmitter zu analysieren und unterschätzen das anatomische Ausmaß der Schädigung. Der verringerte 5HT-Spiegel im anterioren Vorderhirn führte hier zu moderaten anxiogenen Effekten im erhöhten Plus-Labyrinth (elevated plus-maze, EPM) und im Offenfeld (open field, OF). Darüber hinaus zeigten sich Effekte in einem weitern Verhaltensmaß für Angst, der Ultraschallvokalisation (ultrasonic vocalisation, USV). Eine intrastriatale Injektion des $5HT_{2C}$-Antagonisten RS102221 führte dagegen kaum zu Auswirkungen im

Angstverhalten im OF, was darauf hindeutet, dass die anxiogenen Effekte in der vorangegangenen Läsionsstudie eventuell auf andere 5HT-Rezeptortypen oder extrastriatale 5HT-Schädigungen zurückzuführen sind. Untersuchungen von MDMA-Langzeiteffekten unter Berücksichtigung individueller Ängstlichkeit zeigten, dass die Wirkung von MDMA von der vorherigen natürlichen Ängstlichkeit der Ratten abhängig ist. Das Verhalten im EPM, OF und bei der Exploration eines neuen Objektes war nur bei niedrigängstlichen Tieren durch MDMA verändert.

Zusammenfassend lässt sich sagen, dass eine Injektion des Toxins 5,7-DHT in eine bestimmte Hirnregion zu einer weitreichenderen Läsion führen kann und auch andere Hirnareale und Neurotransmitter untersucht werden müssen, um nicht zu einer falschen Schlussfolgerung zwischen Neurochemie und Angst zu gelangen. Zudem reagieren nicht alle Individuen auf eine Manipulation des 5HT-Systems gleich. Daher sollten Versuchstiere vorher auf ihre individuellen Dispositionen, beispielsweise hinsichtlich Angst, untersucht werden. Weiterhin empfiehlt es sich, nicht nur das augenscheinliche Verhalten der Tiere auszuwerten, sondern den Tieren auch “zuzuhören“, da das Verhalten von Ratten nicht nur auf sichtbare Variablen wie beispielsweise Lokomotion oder Vermeidung beschränkt ist, sondern sich eine Manipulation des 5HT-Systems auch auf deren USV auswirkt.

2. Einleitung

2.1 Serotonin

Der Neurotransmitter Serotonin (5-Hydroxytryptamin, 5HT) ist ein Indolamin und gehört somit zu den biogenen Aminen. 5HT kann die Blut-Hirn-Schranke aufgrund seiner hydrophilen Eigenschaften nicht überwinden und wird im Zytoplasma der Neurone durch die Enzyme Tryptophan-Hydroxylase und 5-Hydroxytryptophan-Decarboxylase aus der essentiellen Aminosäure Tryptophan gebildet. Nach der Freisetzung aus der Zelle wird 5HT über ein, in der präsynaptischen Membran lokalisiertes, Transporterprotein (Serotonintransporter, SERT) wieder in das Neuron aufgenommen (Hennig & Netter 2005, Meyer & Quenzer 2005) oder durch das mitochondriale Enzym Monoaminoxidase (MAO) und das Enzym Aldehyddehydrogenase zu 5-Hydroxyindolessigsäure (5HIAA) abgebaut.

Entdeckt und strukturchemisch definiert wurde 5HT erstmals 1948 (Rapport et al. 1948). Aufgrund seiner Gewinnung aus Blutserum und seiner tonisierenden Wirkung auf Blutgefäße wurde dieser Stoff “Serotonin“ genannt. 1953 hat man 5HT auch im zentralen Nervensystem (ZNS) nachgewiesen (Twarog & Page 1953) und 1957 erstmals die Möglichkeit einer Neurotransmitterfunktion diskutiert (Brody & Shore 1957). Heute ist bekannt, dass 5HT in Thrombozyten, der Darmschleimhaut und im ZNS gespeichert wird. Peripher bewirkt 5HT eine Verengung der Blutgefäße, eine Steigerung der Pumpleistung des Herzens, sowie eine Hemmung der Magen- und Dickdarmbewegungen und eine Förderung der Verdauungstätigkeit des Dünndarms. Im ZNS ist 5HT an vielerlei Funktionen, wie Motorik, Thermoregulation, Steuerung des Schlafrhythmus, des Sexualverhaltens und des Appetits, Verarbeitung von Schmerz und höheren kognitiven Funktionen, Modulation von Aggression, Motivation und Emotion und der Kontrolle von Impulsivität beteiligt (Feldman et al. 1997, Schneider & Schmalt 2000, Hennig & Netter 2005). Ein Ungleichgewicht im 5HT-System geht oft mit neuropsychiatrischen Erkrankungen wie Angststörungen, Depression, Schizophrenie und Zwangsstörungen einher (Meyer & Quenzer 2005). Bei der pharmakologischen Behandlung von Depressionen haben sich selektive 5HT-Wiederaufnahmehemmer (selective serotonin reuptake inhibitors, SSRI) und MAO-Hemmer als effektiv erwiesen. SSRI führen dazu, dass 5HT länger im synaptischen Spalt zur Verfügung steht, während MAO-Hemmer den Abbau von 5HT

inhibieren. Solche Antidepressiva erwiesen sich auch gegen verschiedene Angststörungen als wirksam (Blier & de Montigny 1999, Feighner 1999).
Dass 5HT an so vielen verschiedenen Funktionen beteiligt ist, kann vor allem daran liegen, dass das 5HT-System eine große Dichte an Projektionen besitzt (Feldman et al. 1997) und diese in fast alle Hirnregionen und ins Rückenmark ziehen.

2.1.1 Serotonerge Projektionen im ZNS

Die Fasern des 5HT-Systems entspringen hauptsächlich den Raphé-Kernen, die im Hirnstamm liegen. Erstmals beschrieben wurden die Raphé-Kerne um 1900 (Koelliker 1891, Cajal & Ramon 1911). Die Verteilung der 5HT-Neurone im ZNS von Säugern wurde 1964 klassifiziert (Dahlström & Fuxe 1964). Die Projektionen aus den Raphé-Kernen ziehen praktische durch das gesamte ZNS. Die 5HT-Innervation ist dabei selektiv für spezifische Strukturen.
Man unterteilt die Raphé-Kerne in eine kaudale und eine rostrale Zellgruppe (Baumgarten 1991, Jacobs & Azmitia 1992). Die kaudale Zellgruppe liegt in der Medulla oblongata und projiziert in Rückenmark, Hirnstamm und Kleinhirn (Halliday et al. 1995). Zu ihr gehören die Nuclei raphé magnus, raphé obscurus und raphé pallidus. Diese Kerne beeinflussen Motorik, Schmerzmodulation, Respiration und kardiovaskuläre Aktivitäten. Die rostrale Zellgruppe liegt in Pons und Mesenzephalon und projiziert in Telencephalon und Diencephalon (Halliday et al. 1995). Zu ihr gehören die Nuclei raphé dorsalis (DRN), raphé medianus (MRN) und linearis caudalis. Diese Kerne beeinflussen unter anderem emotionale Vorgänge. Die DRN innervieren das laterale Vorderhirn, die MRN hingegen das mediale Vorderhirn, wobei viele Gebiete überlappende Projektionen von DRN und MRN erhalten (Baumgarten 1991, Baumgarten & Grozdanovic 1997). Dennoch kann man DRN- und MRN-Fasern, anhand ihrer morphologischen Unterschiede, voneinander trennen. Neurone mit sehr feinen, stark verzweigten Axonen und kleinen (<1µm), spindelförmigen Varikositäten (Kosofsky & Molliver 1987, Feldman et al. 1997) stammen aus den DRN. Neurone mit dicken Axonen und großen (>2µm), kugelförmigen Varikositäten (Kosofsky & Molliver 1987, Feldman et al. 1997) kommen hingegen hauptsächlich aus den MRN. Zusätzlich entstammt ein kleiner Prozentsatz des feinen 5HT-Fasertyps ebenfalls aus den MRN (Halasy et al. 1992).

Außer den weitreichenden Projektionen des 5HT-Systems könnte auch die große Anzahl an verschiedenen 5HT-Rezeptorsubtypen (Lesch et al. 2003, Hennig & Netter 2005) ein Grund für die umfassenden Funktionen sein, an denen 5HT beteiligt ist.

2.1.2 Serotonerge Rezeptoren im ZNS

Aus den Neuronen freigesetztes 5HT bindet an 5HT-Rezeptoren, die in hoher Dichte im ZNS, Gastrointestinaltrakt, Herz-Kreislaufsystem und im Blut zu finden sind. Es gibt mindestens sieben verschiedene 5HT-Rezeptor-Subfamilien und je nach Rezeptorart hat eine Aktivierung durch 5HT unterschiedliche Auswirkungen.

5HT-Rezeptoren wurden nach Bindungsstudien 1979 in $5HT_1$-Rezeptoren und $5HT_2$-Rezeptoren unterschieden (Peroutka & Snyder 1979), wobei $5HT_1$-Rezeptoren wiederum in $5HT_{1A}$- und $5HT_{1B}$-Rezeptoren unterteilt wurden (Pedigo et al. 1981). Der später entdeckte $5HT_{1C}$-Rezeptor unterschied sich in pharmakologischen Charakteristika von den übrigen $5HT_1$-Rezeptoren (Pazos et al. 1984). Der $5HT_{1D}$-Rezeptor (Heuring & Peroutka 1987), kommt ausschließlich in Spezies vor, die keine $5HT_{1B}$-Rezeptoren enthalten, wie beispielsweise Schwein, Meerschweinchen und Mensch. Weiterhin wurden die Subtypen $5HT_{1E}$ (Leonhardt et al. 1989) und $5HT_{1F}$ (Amlaiky et al. 1992, Adham et al. 1993) klassifiziert. Die $5HT_2$-Rezeptoren werden in den $5HT_{2A}$- (Mengod et al. 1990) und $5HT_{2B}$-Rezeptor (Kursar et al. 1992, Foguet et al. 1992) unterteilt. Die Untersuchung der Aminosäuresequenzen der verschiedenen Rezeptoren führte zu einer Neuklassifizierung des $5HT_{1C}$-Rezeptors in $5HT_{2C}$-Rezeptor. Weiterhin wurden der $5HT_3$- (Fozard 1984), $5HT_4$- (Dumuis et al. 1988), $5HT_5$- (Plassat et al. 1992, Erlander et al. 1993), $5HT_6$- (Monsma et al. 1993, Ruat et al. 1993) und der $5HT_7$-Rezeptor (Lovenberg et al. 1993, Plassat et al. 1993) identifiziert. Die $5HT_5$-Rezeptoren können wiederum in die Subtypen $5HT_{5A}$ und $5HT_{5B}$ unterteilt werden (Hennig & Netter 2005).

Die 5HT-Rezeptoren gehören zur Gruppe der G-Protein-gekoppelten Rezeptoren. Der $5HT_3$-Rezeptor bildet als ligandengesteuerter Ionenkanal die einzige Ausnahme.

Die verschiedenen 5HT-Rezeptorsubtypen stellen im ZNS hauptsächlich postsynaptische Rezeptoren dar. Der $5HT_{1A}$-Rezeptor ist jedoch auch ein somatodentritischer Autorezeptor, während der $5HT_{1B}$- und analog der $5HT_{1D}$-Rezeptor als präsynaptische Autorezeptoren fungieren (Feldman et al. 1997, Hennig & Netter 2005).

Die verschiedenen 5HT-Rezeptorsubtypen sind an unterschiedlichen Verhaltensweisen beteiligt. Bisher am besten untersucht ist der $5HT_{1A}$-Rezeptor, der unter anderem bei Angst und Depression eine Rolle spielt. Die Rezeptoren $5HT_{1B}$ und $5HT_{1D}$ sind hingehen beispielsweise in aggressivem Verhalten involviert. Der $5HT_{2A}$-Rezeptor ist vor allem an neuroendokrinen Funktionen, aber auch an Angst und Schmerz beteiligt. Ein Mangel an hochselektiven Agonisten und Antagonisten führte dazu, dass bisher eine Beteiligung des $5HT_{2C}$-Rezeptor an Motorik, Essverhalten, Hormonsteuerung, Migräne, Angst und Zwangsstörungen diskutiert wird, dies jedoch nicht sicher belegt ist. Der $5HT_3$-Rezeptor ist in Schmerz, Angst- und Essverhalten involviert. Die Funktionen der weiteren 5HT-Rezeptorsubtypen, sind bislang nicht ausführlicher untersucht (Hennig & Netter 2005).

2.1.3 Serotonerge Neurotoxine

Gezielte pharmakologische Neurodegeneration wird in der psychoneurobiologischen Forschung häufig verwendet, um die verschiedenen Funktionen bestimmter Neurotransmitter aufzuzeigen.

1971 wurde erstmals die neurotoxische Wirkung von 5,6-dihydroxyliertem Tryptamin auf das zentrale 5HT-System untersucht (Baumgarten et al. 1971). 5,7-Dihydroxythryptamin (5,7-DHT) erwies sich jedoch als geeigneter, da es eine geringere unspezifische Toxizität besitzt und auch in hohen Dosen von Versuchstieren toleriert wird (Baumgarten & Lachenmayer 1972). Es wird allgemein angenommen, dass 5,7-DHT aufgrund seiner 5HT-ähnlichen Struktur in die 5HT-Zelle aufgenommen wird. Im Neuron wird 5,7-DHT oxidiert, wobei freie Radikale entstehen. Diese Radikale führen zur Denaturierung von Proteinen und bewirken eine Hemmung der Atmungskette (Rotman et al. 1976, Baumgarten & Lachenmayer 2004), so dass es zu einem Absterben der Neurone kommt. Der genaue Mechanismus über den 5,7-DHT wirkt, ist noch nicht vollständig entschlüsselt. Choi et al. (2004) gehen nicht davon aus, dass 5,7-DHT über den SERT in die Zelle aufgenommen wird, da SSRI die Toxizität von 5,7-DHT nicht vermindern. Fest steht, dass 5,7-DHT, in die Ventrikel oder die Raphé-Kerne injiziert, zu einer lang anhaltenden und neurochemisch selektiven, anatomisch jedoch recht globalen Zerstörung der 5HT-Neurone führt (Briley et al. 1990, Hall et al. 1999, Andrade & Graeff 2001, Rex et al. 2003). Neben seiner toxischen Wirkung auf 5HT-Neurone

verursachte 5,7-DHT auch eine Degeneration noradrenerger (NA) Zellen (Baumgarten & Lachenmayer 1972, Baumgarten et al. 1973). Dieser unerwünschte Effekt kann durch eine Vorbehandlung mit einem NA-Wiederaufnahmehemmer verhindert werden, wobei sich Desipramin als effektiv erwiesen hat (Björklund et al. 1975, Nakazato & Akiyama 1998, Fletcher et al. 1999). Dopaminerge (DA) Neurone werden durch 5,7-DHT laut Literatur nicht oder nur gering geschädigt. So fanden Baumgarten et al. (1973) keine DA-Läsion bei 5,7-DHT Injektion in den Ventrikel, Wuttke et al. (1977) nur geringe DA-Schäden in ventrikelnahen Regionen. Auch eine Läsion der DRN und MRN führt zu keiner erkennbaren Veränderung des zentralen DA-Gehaltes (File et al. 1979, Al-Zaharani et al. 1997).

Ein weiteres potentielles 5HT-Neurotoxin ist 3,4-Methylenedioxymethamphetamin (MDMA). MDMA ist ein Amphetaminderivat und Hauptbestandteil von Ecstasy. 1912 wurde MDMA erstmals synthetisiert (Benzenhöfer & Passie 2006, Freudenmann et al. 2006), jedoch nicht pharmakologisch getestet. Später wurde die empathogene und entaktogene Wirkung von MDMA entdeckt und es daraufhin 1985 durch die Drug Enforcement Administration (DEA) verboten. Dieses Verbot verhinderte jedoch nicht, dass MDMA zu einer populären Party-Droge wurde. Akut stimuliert MDMA die 5HT-Ausschüttung und hemmt seine Wiederaufnahme in die Zelle (Cole & Sumnall 2003, Lyles & Cadet 2003), wodurch bei dem Konsumenten meist milde Euphorie und erhöhte Soziabilität hervorgerufen wird (Liester et al. 1992, Vollenweider et al. 1998). Im Tiermodell konnte jedoch gezeigt werden, dass MDMA bei regelmäßiger oder hoher Dosierung neurotoxischen Eigenschaften entfaltet (Commins et al. 1987, Ricaurte et al. 1992, Lyles & Cadet 2003, Baumann et al. 2007). MDMA wird vom SERT in das Neuron aufgenommen und führt zu einer Degeneration der zentralen 5HT-Fasern (Hatzidimitriou et al. 1999, Callahan et al. 2001, Lyles & Cadet 2003). Diese neurotoxische Wirkung zeigt sich jedoch nur bei systemischer, nicht aber bei intracebraler Injektion von MDMA (Esteban et al. 2001, Nixdorf et al. 2001, Darvesh et al. 2005). Dies lässt vermuten, dass peripher gebildete MDMA-Metabolite zur Bildung von freien Radikalen und diese wiederum zur Denaturierung von Proteinen führen (Colado et al. 1997). Man geht in der Regel davon aus, dass das DA- und NA-System durch MDMA unbeeinträchtigt bleiben, jedoch gibt es auch hier gegenteilige Befunde (Baumann et al. 2007). In Tierversuchen werden meist höhere Dosierungen verwendet, als Menschen einnehmen. Da der neurotoxische Effekt von MDMA stark

von der Dosierung und der Frequenz der Einnahme abhängt (Battaglia et al. 1988, O'Shea et al. 1998), ist nicht klar, wie weit die Literatur der Tiermodelle auf den Menschen übertragbar sind. Jedoch sind Primaten MDMA gegenüber anfälliger als Ratten und diese sind wiederum anfälliger als Mäuse (Battaglia et al. 1988, Green et al. 1995, Boot et al. 2000). Des weiteren gibt es auch bei Mensch Hinweise darauf, dass ein starker MDMA-Konsum zu einem verringerten 5HT-Gehalt führt (Green et al. 1995, Boot et al. 2000).

2.2 Angst

Der Begriff Angst wird oft als Oberbegriff verwendet; man kann die Begriffe Angst und Furcht jedoch auch voneinander trennen. Unter Angst versteht man, bei Trennung der Begriffe, ein allgemein und ungerichtetes, unangenehmes Gefühl, das durch die Wahrnehmung einer potentiellen Bedrohung entsteht. Furcht hingegen ist gegenstandsgerichtet, also auf ein konkretes Objekt oder eine bestimmte Situation bezogenen (Steimer 2002, Pawlak & Weyers 2006). Angst stellt sowohl einen emotionalen Zustand (state), der sich über die Zeit und mit der Stärke der Bedrohung ändert, als auch eine stabile individuelle Eigenschaft (trait) dar (Sandford et al. 2000, Steimer 2002).

Angst als Basisemotion ist eine entwicklungsgeschichtlich sehr ursprüngliche Emotion. Sie ist genetisch bestimmt und wird durch Umwelteinflüsse und Erfahrungen modifiziert (Panksepp 1998, Gross & Hen 2004a). Angst tritt bei einer potentiellen Bedrohung auf und führt zu einer Erhöhung der Herzfrequenz und des Blutdrucks und einer Anspannung der Muskeln. Durch diese physiologischen Veränderungen ist der Körper auf eine Flucht vorbereitet (Gross & Hen 2004b). Somit stellt Angst eine natürliche Warn- und Schutzfunktion dar, die zu einer Vermeidung negativer Situationen führt. Es gibt verschiedene Modelle, die versuchen, das neuronale Angstsystem zu beschreiben. Ein grundlegendes Modell ist das Basic Fear System, das Amygdala, Hypothalamus und Hirnstamm beinhaltet und mit dem Schmerzsystem interagiert (Panksepp 1998). Teilweise wird auch das Corpus striatum zu den neuronalen Strukturen des Angstsystems gezählt (Rosen 2004). Gray entwickelte ein Persönlichkeitsmodell mit unabhängigen Systemen, die von unterschiedlichen Reizen aktiviert werden und verschiedene Verhaltensantworten steuern (Gray 1982). Demnach wird das

Verhaltenshemmsystem (Behavioral Inhibition System, BIS) durch Bestrafung und nach Frustration durch Nichtbelohnung aktiviert, führt zu passiver Vermeidung und ist von der Emotion Angst begleitet, während das Verhaltensaktivierungssystem (Behavioral Approach System, BAS) auf Belohnung und Nichtbestrafung reagiert, Annäherungsverhalten und aktive Vermeidung steuert und die Grundlage für Impulsivität bildet. Gray und McNaughton (2000) überarbeiteten das BIS und BAS und entwickelten ein gemeinsames System, das den Annäherungs-Vermeidungs-Konflikt als wichtigstes Kriterium für eine potenzielle Bedrohung sieht.
Um die neurobiologischen und physiologischen Grundlagen von Angst, neuronale Veränderungen bei Angststörungen oder Wirkungen von Psychopharmaka genauer untersuchen zu können, ist die Forschung an Tiermodellen unumgänglich, da die methodischen Möglichkeiten in der Humanforschung limitiert sind.

2.2.1 Angst im Tiermodell

Wird Angst auf der Ebene des Verhaltens definiert, kann man sie nicht nur dem Menschen, sondern auch Tieren zuschreiben. Angstverhalten ist durch Vermeidung, Flucht oder Abwehr gekennzeichnet. Ob Tiere Angst auch empfinden, kann im Tiermodell jedoch nicht gemessen werden.
Es gibt eine Vielzahl von Angstmodellen, welche in konditionierte und spontane, also unkonditionierte Verhaltenstests unterteilt werden (Griebel 1995). Konditionierte Tests erfassen eine gelernte Reaktion auf einen bestimmten Reiz, wobei es sich in der Regel um aversive elektrische Reize handelt. Unkonditionierte oder ethologische Tests hingegen erheben die natürliche Reaktion eines Tieres in einer definierten Situation (Treit 1985), wobei sie den Konflikt der Versuchstiere zwischen Annäherung und Vermeidung des Reizes nutzen. Tiermodelle werden anhand von Augenscheinvalidität, die die phänomenologische Ähnlichkeit zwischen Modell und Störung betrachtet, Konstruktvalidität, die das theoretische Grundprinzip des Modells bewertet, oder prädiktiver Validität, bei der die Effekte einer Substanz im Modell mit deren klinischer Wirksamkeit verglichen wird, beurteilt (Pawlak & Weyers 2006). Insbesondere bei der Frage nach stabilen Eigenschaften der Versuchstiere ist auch die Konstanz des jeweiligen Verhaltens und damit die Reliabilität des Tests von Bedeutung.

In unkonditionierten Tests gemessenes Angstverhalten kann zwischen einzelnen Individuen einer Gruppe, die sich jedoch bezüglich ihres Stammes, Geschlechts, Alters und den Haltungsbedingungen nicht voneinander unterscheiden, deutlich variieren (Ramos et al. 1997, Schmitt & Hiemke 1998, Schwarting et al. 1998, Blizard & Adams 2002). Da eine Gruppierung nach dieser Ängstlichkeit auch bei einer wiederholten Testung nach längerer Zeitspanne (Cavigelli & McClintock 2003, Schwarting & Pawlak 2004, Ray & Hansen 2005) und in anderen Angstmodellen (Ho et al. 2002, Schwarting & Pawlak 2004, Borta et al. 2006) erhalten bleibt, ist dieses Verhalten als ein Trait, also eine individuelle Eigenschaft der Tiere, zu interpretieren (Dellu et al. 1996, Cools & Gingras 1998). Wie Studien an 5HT-Rezeptor Knock-out Mäusen (Finn et al. 2003, Millan 2003, Gordon & Hen 2004) oder auf Angstverhalten selektiv gezüchteter Ratten (Landgraf & Wigger 2002, 2003) zeigen, sind genetische Faktoren an diesen Unterschieden im Angstverhalten beteiligt. Zudem konnte gezeigt werden, dass sich Tiere mit individueller Ängstlichkeit auch in ihrer Physiologie, wie beispielsweise ihrem 5HT-Gehalt oder ihrer Interleukin-2-mRNA-Expression (Schwarting et al. 1998, Pawlak et al. 2003, 2005), unterscheiden.

2.2.2 Angst und Serotonin

An der Modulierung oder Erzeugung von Emotionen wirken eine Vielzahl von Substanzen mit. Ein wichtiger Botenstoff, der an der Steuerung von Angst beteiligt und in der Pathogenese von Angststörungen involviert ist, ist 5HT (Iversen 1984, Handley & McBlane 1993, Griebel 1995, Handley 1995, Menard & Treit 1999, Naughton et al. 2000, Ressler & Nemeroff 2000, Graeff 2002, Lowry et al. 2005). Ein Ungleichgewicht im zentralen 5HT-System geht mit mentalen Störungen, wie Angststörungen oder Depression, einher (van Praag 1996, Ninan 1999, Meyer & Quenzer 2005). Es hat sich gezeigt, dass Antidepressiva, die auf das 5HT-System wirken, auch Angst reduzieren und bei einigen Angststörungen erfolgreich eingesetzt werden können (Gammans et al. 1992, Feighner 1999, Ninan 1999). Die genauen Ursachen von Angsterkrankungen und die Funktion von 5HT in diesem Zusammenhang sind jedoch noch nicht vollständig geklärt. Man vermutet, dass bei Depression und Angsterkrankungen ein 5HT-Mangel zu einer zu schnellen 5HT-Wiederaufnahme in die Zelle führt. Antidepressiva oder Anxiolytika bewirken, dass 5HT länger im synaptischen Spalt verbleibt. Durch diese 5HT-Überflutung wird die

kompensatorische 5HT-Wiederaufnahme normalisiert und die Autorezeptoren der präsynaptischen Membran werden vermehrt stimmuliert und langfristig desensitiviert. Der zentrale 5HT-Stoffwechsel wird so wieder ins Gleichgewicht gebracht.
Tiermodelle ergaben einerseits, dass eine verringerte 5HT-Ausschüttung generell eine Angst mindernde Wirkung zu haben scheint, während Stress und Angst zu einer gesteigerten 5HT-Freisetzung im ZNS führen (Iverson 1984, Briley et al. 1990, Olausson et al. 2001, Rex et al. 2003, Carvalho et al. 2005). Andererseits gibt es hierzu auch gegenteilige Befunde (Harro et al. 2001, Gurtman et al. 2002). Diese Variation in den Ergebnissen kann durch eine Vielzahl von Faktoren, wie der verabreichten Substanz und Dosis, verwendeter Spezies oder den Umgebungsbedingungen, in denen getestet wurde, zustande kommen (Griebel 1995). Zudem sind mehrere 5HT-Rezeptorsubtypen an Angstverhalten beteiligt, wobei einige dieser Rezeptoren eine inverse Wirkung haben (Sánchez 1993, Griebel 1995, Griebel 1996, Panksepp 1998). Insbesondere der $5HT_{1A}$-, $5HT_{2A}$-, $5HT_{2C}$- und der $5HT_3$-Rezeptor werden mit Angst in Zusammenhang gebracht (Griebel 1996, Hennig & Netter 2005). Auch der $5HT_6$-Rezeptor wird hierbei diskutiert (Otano et al. 1999). Ein weiterer möglicher Faktor für die inkonsistente Literaur im Bereich von Angst und 5HT ist der Injektionsort (Griebel 1995, File et al. 1996, Overstreet et al. 2006). 5HT ist im ZNS weit verbreitet und scheint bei der Modulierung von Angst eine Doppelrolle zu spielen (Deakin & Graeff 1991, Graeff et al. 1997). Demnach fördern aufsteigende Fasern aus DRN zur Amygdala und dem frontalen Kortex konditionierte Angst, während Projektionen aus MRN und DRN zum zentralen Höhlengrau (PAG) unkonditionierte Angst hemmen. Um die neuronalen Grundlagen von Angst vollständig zu begreifen, stellt sich nun die Frage, welche Hirnareale noch an diesem Prozess beteiligt sind. 5HT innerviert verschiedene Strukturen, wie Hippocampus, Amygdala, präfrontalem Kortex und zentralem Höhlengrau, von denen man weiß, dass sie in Angstverhalten innvolviert sind (Rex et al. 1993, File et al. 1996, Fendt & Fanselow 1999, Millan 2003). Als eine weitere möglicherweise relevante Struktur hat sich in diesem Zusammenhang auch das ventrale Striatum, mit dem Nucleus accumbens als Hauptstruktur, erwiesen (Sesack & Pickel 1992, Inoue et al. 1994, Otano et al. 1999, Rosen 2004, Carvalho 2005), unter anderem da Ratten mit individuellen Angstverhalten sich in ihrer ventrostriatalen 5HT-Konzentrationen unterschieden (Schwarting et al. 1998). Das ventrale Striatum

gehört zu den Basalganglien und wird von den DRN und MRN innerviert und als eine Schnittstelle zwischen Motivation und Handlung bezeichnet (Mogenson et al. 1980, Spoont 1992). Solche individuellen Unterschiede im 5HT-System könnten wiederum dazu führen, dass diese Tiere unterschiedlich auf Manipualtionen reagieren und so einen weiteren Grund für die inkonsistente Literatur zu 5HT und Angst darstellen.

2.3 Basalganglien

Die Basalganglien (BG) sind ein subkortikal gelegenes Kerngebiet, dessen Teile anatomisch und funktionell eng miteinander verbunden sind. Zu ihnen gehören Amygdala, Corpus striatum und Globus pallidus. In der modernen Nomenklatur wird die Amygdala nicht mehr zu den BG gezählt, dafür jedoch der Nucleus subthalamicus und die Substantia nigra (Heimer et al. 1995, Trepel 2003). Die BG sind an der Steuerung unterschiedlicher Verhaltensaspekte wie Motorik, Motivation, Emotion und Kognition beteiligt (McDonald & Withe 1993, Graybiel 1997, Redgrave et al. 1999). Zudem sind funktionale Störungen der BG die Ursache neurodegenerativer Erkrankungen wie Morbus Parkinson und Chorea Huntington aber auch psychischer Erkrankungen wie Angststörungen und Depression.

Das Corpus striatum wird aufgrund seiner Afferenzen aus dem gesamten Kortex, dem Thalamus, der Substantia nigra und dem Hirnstamm auch als Eingangsstation der BG bezeichnet. Es liegt seitlich des Thalamus und trägt seinen Namen aufgrund seines gestreiften Aussehens. Diese Streifung kommt durch Fasern der Capsula interna zustande, die das Corpus striatum durchdringen. Im Gegensatz zu der Vielzahl an Afferenzen projiziert das Corpus striatum zu einer eher begrenzten Zahl efferenter Strukturen, wobei seine Hauptprojektionswege auf die BG selbst beschränkt sind (Parent & Hazrati 1995a,b). Die Informationsverarbeitung innerhalb der BG geschieht über zwei Hauptwege. Der direkte Weg verläuft der vom Corpus striatum zur Substantia nigra und Globus pallidus pars internus. Dem indirekte Weg sind Globus pallidus pars externus und Nucleus subthalamicus zwischengeschaltet. Die Balance dieser beiden, gegensätzlich wirkenden Projektionswege wird als Grundlage für eine optimale Informationsverarbeitung und -weiterleitung an die Ausgangsstrukturen angesehen. Die generelle Funktion der BG besteht darin, zwischen den zahlreichen Afferenzen diejenigen Informationen zu selektieren, die in einer bestimmten Situation von Bedeutung sind. Die hierbei aktuell notwendigen

Hirnprozesse werden aktiviert und gleichzeitig werden irrelevante oder inkompatible Prozesse gehemmt, um den Organismus bestmöglich auf die jeweilige Situation reagieren zu lassen (Redgrave et al. 1999). Diese Funktion der BG ist von zentraler Bedeutung für das Überleben des Individuums und den Bestand der Art, was die phylogenetische Konservativität dieses Systems erklärt.

2.3.1 Ventrales Striatum

Das Corpus striatum wird in das dorsale oder Neostriatum und das ventrale Striatum unterteilt (Trepel 2003, Voorn et al. 2004). Das dorsale Striatum setzt sich aus Nucleus caudatus und Putamen zusammen. Nucleus caudatus und Putamen unterscheiden sich trotz ihrer Trennung durch die Capsula interna strukturell und funktionell nur wenig voneinander und entstammen entwicklungsgeschichtlich einer gemeinsamen Anlage. Bei Nagern findet sich die Auftrennung des dorsalen Striatums nicht, da ihnen die Capsula interna fehlt. Hier wird das dorsale Striatum daher gesamtheitlich als Caudato-putamen bezeichnet. Das ventrale Striatum setzt sich aus dem Nucleus accumbens (Nacc) und Teilen des Tuberculum olfactorium zusammen.

Der Nacc bildet den ventrorostralen Bereich des Corpus striatum. Er ist vorwiegend mit motorischem und belohnungsmotiviertem Verhalten assoziiert, hat sich aber auch für Angst als relevant erwiesen (Schwarting et al. 1998, Otano et al. 1999, Rosen 2004). Der Nacc steht mit Hirnarealen in Verbindung, die die Wirkung von Emotion auf Verhalten vermitteln (Kandel et al. 1996, Trepel 2003) und wird als Schnittstelle zwischen Motivation und Handlung gesehen (Mogenson et al. 1980, Spoont 1992, Trepel 2003). Er kann in eine zentrale Kern- (Core) und eine umliegende Schalenregion (Shell) unterteilt werden. Selten wird in der Nomenklatur auch der rostrale Pol, als dritte Region, genannt. Die Projektionen des Nacc sind topographisch organisiert, wobei die Schale limbische Strukturen innerviert, während der Kern zu motorverwandten Regionen der BG projiziert. Zudem werden die verschiedenen Strukturen des Nacc von unterschiedlichen 5HT-Fasertypen innerviert. Die meisten Regionen werden von dünnen glatten Axonen innerviert, während in die caudale Schale dicke Axone mit großen runden Varikositäten projizieren (Brown & Molliver 2000, Lehmann et al. 2003).

3. Fragestellung

Die psychologischen und neurochemischen Grundlagen der Emotion Angst werden in dieser Arbeit anhand von Verhaltenstests, sowie von pharmakologischen, neurotoxischen und -chemischen Methoden untersucht. Besondere Beachtung wird hierbei auf die individuellen Unterschiede der Versuchstiere und die funktionelle Rolle von 5HT, insbesondere im Nacc, gelegt.

Es ist bekannt, dass zentrales 5HT in Angstverhalten involviert ist (Iversen 1984, Griebel 1995, Handley 1995, Menard & Treit 1999, Graeff 2002). Die Literatur ist jedoch inkonsistent und behaviorale Befunde aus Studien, die den Zusammenhang zwischen 5HT und Angst mit neurotoxischer Schädigung untersuchen, sind oft widersprüchlich.

Wie bereits erwähnt, könnte ein Grund hierfür sein, dass das 5HT-System weit verzweigte Projektion besitzt, welche in verschiedenen Hirnarealen gegenteilige Effekte auf Angst bewirken (Deakin & Graeff 1991), die meisten Studien jedoch mit einer generellen Manipulation des zentralen 5HT arbeiten. Es stellt sich also die Frage, ob das, nach 5HT-Manipulation gezeigte, Angstverhalten vom Injektionsort abhängig ist (Griebel 1995, File et al. 1996, Overstreet et al. 2006). Neben anderen, hierfür bekannten Strukturen (Rex et al. 1993, File et al. 1996, Fendt & Fanselow 1999, Millan 2003), könnte auch der Nacc an Angst- und Vermeidungsverhalten beteiligt sein (Sesack & Pickel 1992, Inoue et al. 1994, Schwarting et al. 1998, Otano et al. 1999, Rosen 2004, Carvalho 2005). Ratten, mit differentiellem individuellen Angstverhalten, zeigen unterschiedlich hohe Konzentrationen an 5HT im ventralen Striatum, wohingegen andere Hirnareale oder Neurotransmitter keine Unterschiede aufweisen (Schwarting et al. 1998). Weiterhin führt eine erniedrigte $5HT_6$-Rezeptor-Dichte im Nacc zu einer Erhöhung von Angstverhalten (Otano et al. 1999). Setzt man die Versuchstiere dem Stress eines Angsttests aus, führt dies akut zu einer Abnahme der 5HT-Konzentration im Nacc (Carvalho 2005).

Weiterhin sind mehrere 5HT-Rezeptoren an Angst beteiligt und haben zum Teil gegensätzliche Wirkungen (Sánchez 1993, Griebel 1995, Griebel 1996, Panksepp 1998). Auch hieraus könnten die uneinheitlichen Befunde, aus Studien mit generellen 5HT-Manipulationen, herrühren. Die Rezeptoren $5HT_{1A}$, $5HT_{2A}$, $5HT_{2C}$, $5HT_3$ und $5HT_6$ beeinflussen das Angstverhalten (Sánchez 1993, Rodgers et al. 1995, Griebel

1995, 1996, Panksepp 1998, Otano et al. 1999), wobei der $5HT_{2C}$-Rezeptor hier in wachsendes Interesse zu rücken scheint (Kennett et al. 1997, 2000, Wood et al. 2001, Campbell & Merchant 2003, Alves et al. 2004, Winstanley et al. 2004, Ji et al. 2006). Auch rezeptorspezifische Manipulationen sollten nicht generell, sondern lokal begrenzt vorgenommen werden, da der Substanzeffekt von dem Hirnareal, in dem der Rezeptor lokalisiert ist, abhängt. So hat beispielsweise ein $5HT_{1A}$-Agonist anxiogene Effekte nach Injektion in den dorsalen Hippocampus oder die Amygdala (Andrews et al. 1994, Hogg et al. 1994, File et al. 1996, Gonzalez et al. 1996), aber anxiolytische Effekte nach Injektion in die DRN (Andrews et al. 1994, File et al. 1996, Lanfumey & Hamon 2004). Im Nacc ist der $5HT_{1A}$-Rezeptor kaum vertreten und bewirkt in diesen injiziert keine Veränderung im Angstverhalten (Stefanzki et al. 1993). Ähnliche lokal unterschiedliche Wirkungen könnten erklären, daß auch die Literatur zu $5HT_{2C}$-Rezeptormanipulationen und Angst inkonsistente Ergebnisse aufweist. Systemisch verabreicht, reduziert ein $5HT_{2C}$-Agonist in einigen Studien Angst (Scorza et al. 1996), in anderen jedoch erhöht er Angst (Ji et al. 2006), während ein inverser $5HT_{2C}$-Agonist (Wood et al. 2001) oder ein $5HT_{2C}$-Antagonist (Kennett et al. 1997) Angst reduziert.

Weiterhin können die widersprüchlichen Effekte in der Literatur auf natürliche Unterschiede im Verhalten einzelner Individuen zurückgeführt werden (Ho et al. 2002, Borta et al. 2006). Diese interindividuellen Verhaltensunterschiede spiegeln sich auch in physiologischen Parametern, wie beispielsweise dem 5HT-Gehalt im ventralen Striatum, wider (Schwarting et al. 1998, Pawlak et al. 2003, 2005). Aus anderen Studien ist bereits bekannt, dass sich verschiedene Rattenstämme in ihrem Verhalten in unterschiedlichen Angstmodellen, der 5HT-Ausschüttung unter Stress und der Reaktivität auf Pharmaka, wie beispielsweise Diazepam, unterscheiden (Rex et al. 1996, 1999, Bert et al. 2001). Zudem unterscheiden sich Rattenlinien mit unterschiedlichem endogenen Angstverhalten auch in ihrer Reaktion auf MDMA (Green & McGregor 2002). Solch eine differentielle Reaktivität auf MDMA tritt auch zwischen Ratten gleicher Linie, Alter, Geschlecht und Vorerfahrung jedoch mit unterschiedlichem Angstverhalten auf (Ho et al. 2004). Diese Tatsachen können die widersprüchlichen Effekte aus Untersuchungen zu Angst und Langzeiteffekten von MDMA erklären (Morley et al. 2001, Mechan et al. 2002, Piper 2007). Da MDMA über das 5HT-System wirkt und bei Ratten mit verschieden individuellem Angstverhalten

der Gehalt an zentralem 5HT variiert, ist anzunehmen, dass solche natürlichen Unterschiede im Angstverhalten auch einen Einfluss auf die Langzeitwirkung von MDMA haben.

Insgesamt können also die komplexen Hirn- und Verhaltensmechanismen ein Grund für die inkonsistenten Ergebnisse aus Studien, die sich mit Angst und 5HT beschäftigen, sein. In dieser Arbeit wird daher die lokale Wirkung serotonerger Manipulationen untersucht (Studie 1, 2) und die gegebene individuelle Ängstlichkeit der Versuchstiere mit einbezogen (Studie 3). Untersucht und diskutiert werden die Wirkung des 5HT-Toxins 5,7-DHT, des auf das 5HT-System wirkenden MDMA, sowie des selektiven $5HT_{2C}$-Antagonist RS102221 (Weinhardt et al. 1996, Bonhaus et al. 1997) auf das Anstverhalten von Ratten. Das Neurotoxin 5,7-DHT und der $5HT_{2C}$-Antagonist, wurden jeweils direkt in den Nacc injiziert, um die Rolle von striatalem 5HT auf Angstverhalten zu untersuchen. Die Langzeiteffekte von MDMA wurden unter Berücksichtigung der vorherigen natürlichen Ängstlichkeit der Ratten untersucht, um mögliche Zusammenhänge zwischen Individualität und Drogenwirkung aufzuzeigen.

4. Methoden

Hier sollen nur die wichtigsten Methoden genauer beschrieben werden. Weitere verwendete Verhaltensmodelle sind in den entsprechenden Veröffentlichungen, bzw. Manuskripten zu finden.

4.1 Offenfeld

Das Offenfeld (open field, OF) ist ein Verhaltenstest zur Messung motorischer Aktivität und unkonditioniertem Explorations- sowie Vermeidungsverhalten. Es gehört zu den ältesten (Hall 1934) und am häufigsten verwendeten Methoden in der tierexperimentellen Verhaltensforschung (Prut & Belzung 2003).

Ein OF (Abb. 1) ist eine umrandete und, im Verhältnis zum Heimatkäfig, große Fläche. Das Versuchstier kann die Versuchsapparatur eine definierte Zeit frei explorieren. Das OF dient zum Einen zur Untersuchung des Neugier- oder Explorationsverhaltens, da es bei der ersten Testung eine neue, relativ neutrale Umgebung darstellt. Das Explorationsverhalten von Nagern wird vor allem durch die Häufigkeit, mit der sich die Tiere auf die Hinterbeine aufrichten (rearing) definiert. Zum Anderen misst man im OF die lokomotorische Aktivität des Versuchstieres. Hierbei kann zusätzlich das räumliche Aufenthaltsmuster untersucht werden, indem man die erhobenen Verhaltensparameter im Zentrum und im Rand des OF differenziert betrachtet. Vermehrter Aufenthalt im Zentrum wird als Maß für verringertes Vermeidungs- oder Angstverhalten herangezogen, da das Zentrum eine freie Fläche darstellt, welche von den Tieren natürlicherweise gemieden wird, und der Rand im Gegensatz hierzu an schützenden Wänden liegt. Pharmakologisch als Angstmodell vallidiert wurde das OF mithilfe von Anxiolytika, da diese bewirken, dass sich Ratten vermehrt in das aversive Zentrum begeben (Treit & Fundytus 1988, Belzung & Le 1994, Prut & Belzung 2003). Generell ist zu berücksichtigen, dass Form, Größe und Beleuchtung des OF-Tests in vielen Studien stark variieren, wodurch das Verhalten der Tiere unterschiedlich beeinflusst werden kann (Valle 1970, Prut & Belzung 2003). Die Aufteilung in Zentrum und Rand, und in diesem Zusammenhang die Messung von Angst- oder Vermeidungsverhalten im OF, ist nur dann sinnvoll, wenn das OF groß genug und relativ hell ausgeleuchtet ist, so dass das Zentrum von den Tieren auch als aversiv wahrgenommen wird.

In den Studien dieser Dissertation wurden verschiedene Variationen des OF-Tests verwendet. In den Studien 1 und 3 wurden die Versuchstiere in einem relativ kleinen OF (Abb. 1, links) getestet. Dieses ist mit einem, auf Lichtschranken basierenden, automatischen Auswertungsprogramm (TruScan, Coulbourn Instruments, USA) versehen und ermöglicht unter anderem die automatische Auswertung des Aufrichtverhaltens. Das Aufrichtverhalten war in diesen Studien von Interesse, da es durch die akute Wirkung von MDMA, welches den Tieren in diesen Studien verabreicht wurde, beeinflusst werden kann. In der Studie 2 wurden die Versuchstiere in einem große OF (Abb. 1, rechts) getestet, da der OF-Test in dieser Studie als einziges Angstmodell eingesetzt wurde und als solches sichergestellt sein sollte.

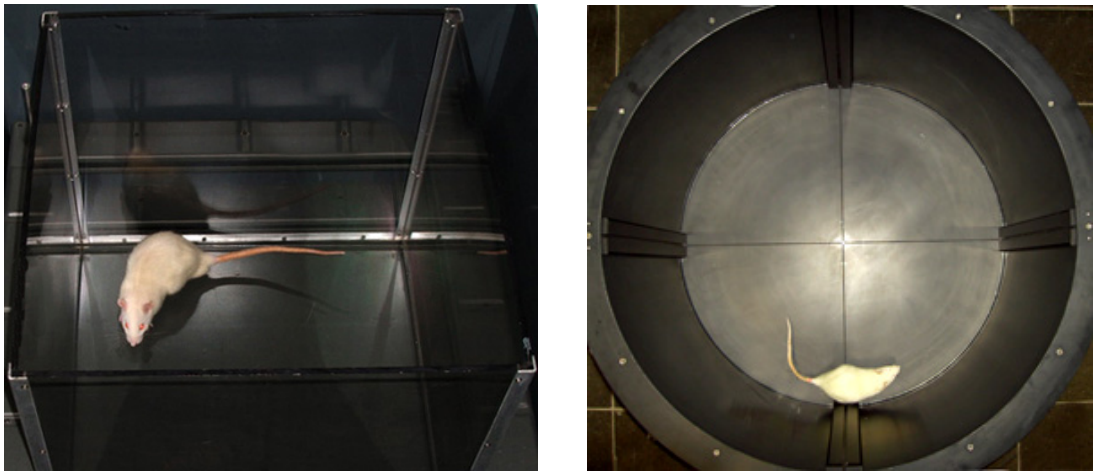

Abbildung 1: Offenfeld (OF)
Links: kleiner OF-Test für Ratten der Firma Coulbourn Instruments; Rechts: großer OF-Test für Ratten

4.2 Erhöhtes Plus-Labyrinth

Das erhöhte Plus-Labyrinth (elevated plus-maze, EPM) ist einer der am weitesten verbreiteten und auf der Basis von Verhalten, Pharmakologie und Neurobiologie am besten untersuchtesten Modelle zur Messung von Angstverhalten, speziell bei Nagern (Carobrez & Bertoglio 2005).

Das EPM (Abb. 2) basiert auf dem X-Maze von Montgomery (1955), wurde jedoch erst später validiert (Handley & Mithani 1984, Pellow et al. 1985). Das EPM ist vom Boden erhöht und besteht aus zwei gegenüberliegenden geschützten, von Wänden umschlossenen und zwei ungeschützten, offenen Armen und kann vom Versuchtier frei exploriert werden. Unbehandelte Tiere zeigen eine deutliche Präferenz für die geschlossenen Arme, was durch die natürliche Angst der Tiere vor offenen Flächen, Helligkeit und Höhen erklärt wird (Treit et al. 1993, Dawson & Tricklebank 1995, Finn et al. 2003). Substanzen, die beim Menschen Angst induzieren, reduzieren die Aufenthaltsdauer und die Eintritte in die offenen Arme und umgekehrt erhöhen klinisch effektive Anxiolytika die Präferenz für die offenen Arme (Pellow et al. 1985, Pellow & File 1986, Rodgers et al. 1997). Aufgrund dieser Zusammenhänge wird eine Vermeidung der offenen Arme im EPM als Angstverhalten interpretiert. Tiere, die sich häufig auf den offenen Armen aufhalten (high open arm, HOA), werden als nicht oder wenig ängstlich bezeichnet (low anxiety, LA), wohingegen Tiere, die sich selten auf den offenen Armen aufhalten (low open arm, LOA), als ängstlich bezeichnet werden (high anxiety, HA). Die gesamten Armeintritte sind ein Maß für die allgemeine Aktivität und werden mit den Eintritten in die offenen Arme in Beziehung gesetzt, um eine reduzierte Lokomotion nicht fälschlicherweise als anxiogene Wirkung zu interpretieren.

Der EPM-Test wird meist an zwei aufeinanderfolgenden Tagen durchgeführt. Am ersten Tag ist die Apparatur für die Tiere völlig neu, wohingegen sie am zweiten Tag bereits bekannt ist. Die Versuchstiere meiden die offenen Arme am zweiten Versuchstag stärker als am ersten, wobei jedoch die Rangreihenfolge zwischen den Tieren erhalten bleibt. Eine längere Pause zwischen zwei Testdurchführungen hebt den Habituationseffekt wieder auf und führt im zweiten Test zu ähnlichen Aufenthaltszeiten wie im ersten Test (Schwarting & Pawlak 2004).

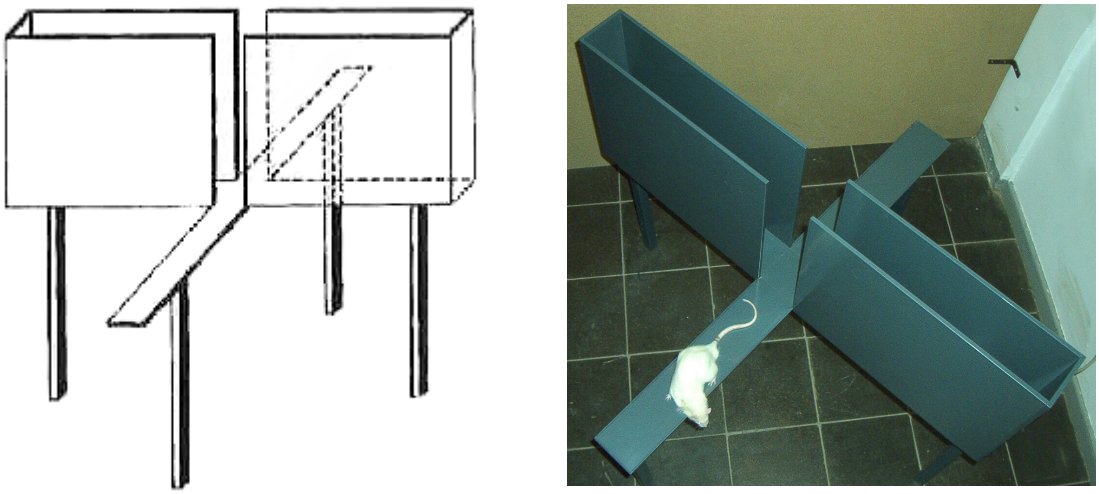

Abbildung 2: erhöhtes Plus-Labyrith (EPM)
Links: Skizze eines EPM; Rechts: EPM-Test für Ratten

4.3 Stereotaxie und Mikroinjektion

Die präzise Mikroinjektion von Pharmaka ins Gehirn ist eine geeignete Methode, um den Zusammenhang zwischen Neurochemie und Verhalten zu analysieren.
Für eine Mikroinjektion in ein bestimmtes Hirnareal wird zuvor eine Kanüle, durch eine stereotaktische Operation, in das entsprechenden Gebiet des Gehirns eingeführt (Abb. 3, links). Hierfür werden mithilfe eines stereotaktischen Atlas (z.B. Paxinos & Watson 1997), die dreidimensionalen Koordinaten des Zielgebietes bestimmt, die sich auf einen Referenzpunkt am Schädel, meist Bregma, beziehen. Der Schädel des narkotisierten Versuchstieres wird in einem stereotaktischen Apparat plan fixiert, Bregma freigelegt und kleine Löcher über dem Zielgebiet gebohrt. Eine Kanüle wird, entsprechend der Koordinaten des Atlas, ortgenau in das Gehirn abgesenkt. Eine langfristig wirksame Substanz wird nun direkt in das ausgewählte Areal injiziert, oder die Kanüle am Schädel des Tieres fixiert und eine akut wirkende Substanz kurz vor dem Verhaltenstest über diese Kanüle appliziert.
Die Injektion von Substanzen ins Gehirn erfolgt in sehr geringen Volumen und in definierter und gleichmäßiger Geschwindigkeit. Um diese Mikroinjektion (Abb. 3, rechts) präzise auszuführen, bedient man sich einer Mikroinjektionspumpe, in die eine Spritze eingespannt wird. Diese Spritze ist über einen sehr dünnen Schlauch mit einer Injektionskanüle verbunden. Das gesamte System wird mit sterilem Wasser

befüllt und die zu injizierende Substanz, von der Injektionskanüle aus, in den vorderen Teil des Schlauchs aufgezogen. Wasser und Injektionslösung werden voneinander durch eine Luftblase oder eine farbige Substanz, die sich mit den anderen Lösungen nicht vermischt, getrennt. Die Injektionskanüle wird in die bereits implantierte Kanüle, die sogenannte Führungskanüle, eingeführt (Peterson 1998). Nachdem sich die Injektionskanüle am Zielort befindet wird, eine geringe, genau definierte Menge der zu injizierenden Substanz über die Pumpe und das Schlauchsystem automatisch, langsam und kontinuierlich in das Gehirn des Versuchstieres appliziert. Hierbei kann die Luftblase oder farbige Lösung in ihrer Bewegung beobachtet werden, um das gleichmäßige Injizieren zu überwachen.

Abbildung 3: Stereotaxie & Mikroinjektion
Links: stereotaktische Implantation einer Kanüle; Rechts: schematische Darstellung der Mikroinjektion mittels Mikroinjektionspumpe

4.4 Neurochemische Analyse

Die neurochemischen Analysen wurden post mortem mittels Hochdruck-Flüssigkeits-Chromatographie (High Pressure/Performance Liquid Chromatography, HPLC) mit elektrochemischer Detektion (HPLC-EC) durchgeführt. Mit dieser Methode kann der Neurotransmittergehalt in verschieden Hirngebieten gemessen und so die chemische Zusammensetzung von einzelnen Arealen des Gehirns, sowie Zusammenhänge zwischen Neurochemie und Verhalten untersucht werden.

Für die HPLC-Analyse müssen die entnommenen Gewebeproben folgendermaßen aufbereitet werden (Abb. 4, links). Das, in einem Antioxidans aufgefangene, Gewebe wird homogenisiert, um die Zellmembranen aufzubrechen, und zentrifugiert, um die schweren Zellmembranreste von dem leichten Überstand zu trennen. Der Überstand wird anschließend gefiltert und kann dann mittels HPLC-Verfahren analysiert oder bei –80°C aufbewahrt werden.

Das Verfahren der HPLC-Analyse (Abb. 4, rechts) setzt sich aus zwei Teilschritten zusammen, der Chromatographie und der eigentlichen Substanzanalyse. Bei der Chromatographie wird ein Stoffgemisch zwischen zwei Phasen, der mobilen und der stationären Phase, in seine einzelnen Bestandteile aufgetrennt. Die Verweildauer (Retentionszeit) der einzelnen Probenbestandteile in der stationären Phase variiert aufgrund deren unterschiedlichen Charakteristika, wie beispielsweise Molekülgröße, Ladung oder Wechselwirkungen, und führt so zur Trennung der einzelnen Substanzen. Es gibt verschiedene Chromatographieverfahren. In dieser Arbeit wurde die Umkehrphasen-Verteilungs-Chromatographie (Reversed Phase, RP) verwendet, bei der die Trennung der Substanzen durch deren unterschiedliche Löslichkeit in den Phasen erfolgt. Die RP-HPLC ist die gängigste Methode der analytischen HPLC-Trennungen. Mit Hilfe von Standardlösungen, welche jeweils nur eine Substanz enthalten, wird die Retentionszeiten der zu erwartenden Probenbestandteile bestimmt, um eine eindeutige Zuordnung treffen zu können.

In der anschließenden Analyse wird die Konzentration der jeweiligen isolierten Substanzen durch einen Detektor erhoben. Es gibt verschiedene Detektionsmethoden. Der hier verwendete elektrochemische Detektor (electrochemical detection, EC) misst die Änderung eines angelegten Stromflusses, die durch Oxidation oder Reduktion der zu messenden Stoffe verursacht wird. Mit Hilfe der Standardlösungen, welche eine definierte Menge der einzelnen Substanzen

enthalten, kann die quantitative Substanzmenge in der Probe bestimmt werden, da die Veränderung des Stromflusses proportional zur Menge der oxidierten oder reduzierten Substanz ist. Zusätzlich zu diesem externen Standard wird der zu analysierenden Probe ein interner Standard (meist einem der zu messenden Neurotransmitter chemisch ähnliche Substanz) zugefügt, der dieselben Aufbereitungsschritte, wie die zu messende Substanz, durchläuft. Um Schwankungen auszugleichen, wird die Konzentrationen der Probenbestandteile in Relation zu diesem internen Standard berechnet.

Neben dem Gewebegehalt der verschiedenen Neurotransmitter wird auch der Gehalt ihrer spezifischen Metabolite bestimmt. Das Verhältnis zwischen Metabolit und Transmitter wird häufig als Hinweis auf die Aktivität des Neurons genutzt. Die Methode der post mortem HPLC hat gegenüber der Mikrodialyse (bei der einem lebenden Tier extrazelluläre Proben aus einem bestimmten Hirnareal entnommen werden) den Vorteil, dass viele Hirnregionen auf ihren Transmittergehalt hin analysiert werden können.

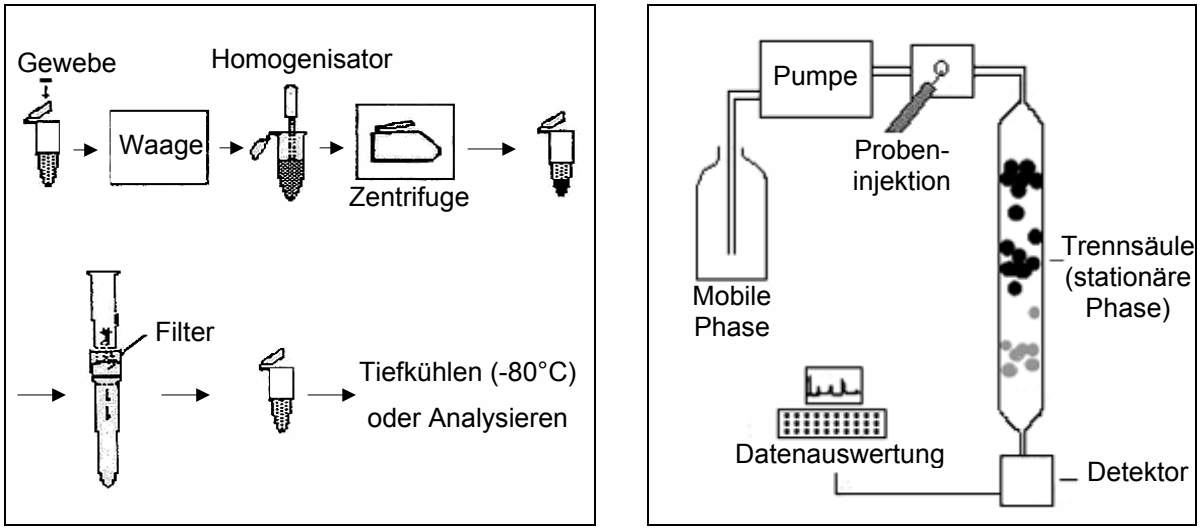

Abbildung 4: High Pressure Liquid Chromatography (HPLC)
Links: Probenaufbereitung für die post mortem HPLC-Analyse; Rechts: schematische Darstellung der technischen Bestandteile des HPLC-Verfahrens

5. Durchgeführte Studien

Die vollständigen veröffentlichten Studien, bzw. die zur Veröffentlichung eingereichten Manuskripte sind im Anhang dieser Arbeit eingebunden.

5.1 Neurochemical and behavioral consequences of striatal injection of 5,7-DHT

Es ist bekannt, dass 5HT an der Modulation von Angstverhalten beteiligt ist (Iversen 1984, Handley & McBlane 1993, Griebel 1995, Handley 1995, Menard & Treit 1999, Graeff 2002, Lowry et al. 2005). Studien, die Angstverhalten in Nagern nach 5HT Läsion durch 5,7-DHT untersuchen, injizieren das Toxin jedoch meist in die Ventrikel oder die Raphé-Kerne, was zu einer generellen Läsion von zentralem 5HT führt (Briley et al. 1990, Hall et al.1999, Andrade & Graeff 2001, Rex et al. 2003). Die Verhaltenseffekte solcher Studien sind oft inkonsistent. Da es Hinweise auf unterschiedliche Effekte von 5HT in verschiedenen Hirnarealen gibt (Deakin & Graeff 1991) und sich der Nacc hier als interessant erwiesen hat (Sesack & Pickel 1992, Inoue et al. 1994, Schwarting et al. 1998, Otano et al. 1999, Rosen 2004, Carvalho 2005), wurden in dieser Studie die Effekte auf Angstverhalten nach einer Injektion von 5,7-DHT in das ventrale Striatum untersucht. Es konnte gezeigt werden, dass diese Manipulation zu einer dosisabhängigen Reduktion von 5HT im ventralen Striatum, Neostriatum, frontalen Kortex und der Amygdala führt. Diese Läsion war im anterioren Vorderhirn (Striatum und frontaler Kortex) deutlicher ausgeprägt als in der Amygdala. Zusätzlich konnte gezeigt werden, dass eine Vorbehandlung mit dem, in der Literatur fast ausschließlich verwendeten, NA-Wiederaufnahmehemmer Desipramin nicht ausreicht, da DA durch die Injektion von 5,7-DHT ins ventrale Striatum dort ebenfalls reduziert war. Diese DA-Läsion konnte durch zusätzliche Vorbehandlung mit dem DA-Wiederaufnahmehemmer Nomifensin verhindert werden. Bei der Analyse der Verhaltenseffekte solcher neurochemisch spezifischen 5HT-Läsionen zeigten sich Hinweise auf anxiogene Effekte im EPM und im OF. Im aktiven Vermeidungsverhalten traten keine Läsionseffekte auf, die Ultraschallvokalisation (ultrasonic vocalisation, USV) der Ratten während diesem Test indessen war dennoch beeinflusst. Neben für den Menschen hörbaren Tönen, geben Ratten in aversiv motivierten Situationen USV-Rufe im 22kHz-Bereich von sich (Tonoue et al.

1986, Sánchez 2003, Jelen et al. 2003). Diese 22kHz-Rufe können als weiteres Verhaltensmaß und Indikator für Angst herangezogen werden (Wöhr et al. 2005), da sie den affektiven Zustand der Tiere erfassen und beispielsweise individuelle Unterschiede im EPM mit 22kHz-USV assoziiert sind (Borta et al. 2006). In dieser Studie zeigte sich, dass die 5HT-Läsion eine Auswirkung auf die maximale Ruffrequenz, also die Tonhöhe, und die Frequenzbandbreite jeweils an den Rufenden der schockinduzierten 22kHz-USV hat. Borta et al. (2006) fanden Unterschiede in ähnlichen Frequenzparameter in der USV unbehandelten Ratten mit unterschiedlichem Angstverhalten. Das ansonsten unauffällige OF-Verhalten der lädierten Tiere unterschied sich unter zusätzlicher Gabe von MDMA deutlich von dem der Kontrolltiere. Akut stimuliert MDMA die 5HT-Ausschüttung und hemmt seine Wiederaufnahme in die Zelle (Cole & Sumnall 2003, Lyles & Cadet 2003), was das 5HT-System der lädierten Tiere zusätzlich aus dem Gleichgewicht bringt. In den Kontrolltieren erhöhte MDMA die Lokomotion und Zentrumseintritte, diese Aktivierung war in den lädierten Tieren weniger stark ausgeprägt.

Da die 5HT-Läsion nicht allein das ventrale Striatum, sondern allgemein das anteriore Vorderhirn betraf, können die gefundenen Verhaltenseffekte nicht eindeutig auf die funktionale Rolle von 5HT im ventralen Striatum zurückgeführt werden. In einer weiteren Studie wurde deshalb mit einem 5HT-Antagonisten gearbeitet und dieser direkt ins ventrale Striatum injiziert.

5.2 Striatal injection of a $5HT_{2C}$ antagonist and the consequences in OF behavior

Diese Studie wurde bisher nicht zur Veröffentlichung eingereicht, daher befindet sich im Anhang kein zugehöriges Manuskript und die Studie wird in diesem Teil etwas ausführlicher beschrieben.

Es gibt mehrere 5HT-Rezeptorsubtypen, die bekanntermaßen an Angstverhalten beteiligt sind (Sánchez 1993, Rodgers et al. 1995, Griebel 1995, 1996, Panksepp 1998, Otano et al. 1999). Zu diesen gehören auch die postsynaptischen Rezeptoren $5HT_{2A}$ und $5HT_{2C}$, die im Nacc dicht lokalisiert sind (Abramowski 1995, Compan et al. 1998, Clemett et al. 2000). $5HT_{2A}$- und $5HT_{2C}$-Rezeptoren haben jedoch teilweise gegenteilige Effekte (Rodgers et al. 1995, Millan et al. 1998, Fletcher et al. 2002, Winstanley et al. 2004). Der $5HT_{2C}$-Rezeptor ist im Zusammenhang mit Angst in den

letzten Jahren immer interessanter geworden (Kennett et al. 1997, 2000, Wood et al. 2001, Campbell & Merchant 2003, Alves et al. 2004, Winstanley et al. 2004, Ji et al. 2006). Zudem haben $5HT_{2C}$-Antagonisten ähnliche Effekte wie 5HT-Läsionen (Winstanley et al. 2004).

Um die funktionale Rolle des $5HT_{2C}$-Rezeptors im Nacc auf Angstverhalten zu untersuchen, wurden die Verhaltenseffekte in einem großen OF nach Mikroinjektion des selektiven $5HT_{2C}$-Antagonist RS102221 (Weinhardt et al. 1996, Bonhaus et al. 1997) in das ventrale Striatum analysiert. Männlichen Wistar Ratten (Harlan Winkelmann, Deutschland) wurden bilaterale Führungskanülen (26gauge ≈ Ø 0,46mm; Plastics One, USA) in den Nacc (Paxinos & Watson 1997) implantiert. Eine Woche nach der Operation wurden den Ratten verschiedene Dosierungen des $5HT_{2C}$-Antagonisten RS102221 bilateral über Injektionskanülen (33gauge ≈ Ø 0,2mm; Plastics One, USA) verabreicht und die Tiere konnten das OF (Ø 79cm, Weißlicht 30lux) für 45Minuten frei explorieren. Für die Auswertung wurde die Versuchsapparatur in ein Zentrum (Ø 59,25cm) und eine äußere Randzone aufgeteilt. Lokomotion, Zoneneintritte und -aufenthaltszeit wurden mit Hilfe eines automatischen Auswertungsprogramms (Etho-Vision Pro 3.0, Noldus, Germany) analysiert. Während der Testung wurde zusätzlich die USV der Tiere mit einem Avisoft-Recorder aufgezeichnet (Avisoft Bioacoustics, Deutschland; Einstellungen siehe Ludwig & Schwarting 2007), da 5HT-Manipulationen die USV von Ratten beeinflussen können (Jolas et al. 1995, Sánchez 2003, Campbell & Merchant 2003, Ludwig & Schwarting 2007). Zur histologischen Verifizierung der Position der Kanüle wurden die Ratten perfundiert und die Gehirne entnommen und analysiert.

In die Auswertung des OF-Verhaltens (zweifaktorielle Varianzanalyse mit Messwiederholung) gingen folgende Gruppengrößen ein: 0,9% Kochsalzlösung (NaCl; n=10), 0,2µg (n=10), 1µg (n=8) und 2µg (n=10) RS102221. Diese Dosierungen des $5HT_{2C}$-Antagonist RS102221 wurden aufgrund von Erfahrungswerten aus der Literatur gewählt (McMahon et al. 2001, Filip & Cunningham 2002, 2003). Es zeigte sich ein tendentieller Zusammenhang (Abb. 5 links, p=.059, 2-seitig) von Dosis und Lokomotion. Die Tiere aus der 1µg RS102221 Gruppe legten eine geringere Strecke zurück als die Ratten der anderen Gruppen. McMahon et al. (2001) fanden keinen Effekt auf spontane Lokomotion nach Injektion des RS102221 in den Nacc, jedoch haben sie mit geringeren Dosierungen (0,05-

0,5µg) gearbeitet. In bezug auf das Angstverhalten, also die Eintritte (nicht gezeigt, p=.357, 2-seitig) und die Aufenthaltszeit (Abb. 5 rechts, p=.601, 2-seitig) im Zentrum, ließ sich keine signifikante Beeinflussung durch den $5HT_{2C}$-Antagonist RS102221 finden. Deskriptiv lässt sich sagen, dass sich die 1µg Gruppe in den ersten 20Minuten vermehrt im Zentrum aufhielt, dies jedoch eventuell durch die großen Standardfehler zu keinem signifikanten Ergebnis führt. Overstreet et al. (2006) injizierten einen inversen $5HT_{2C}$-Agonisten in den Nacc und fanden keinen Hinweis auf eine Beinflußung des Angstverhaltens durch diese Behandlung.

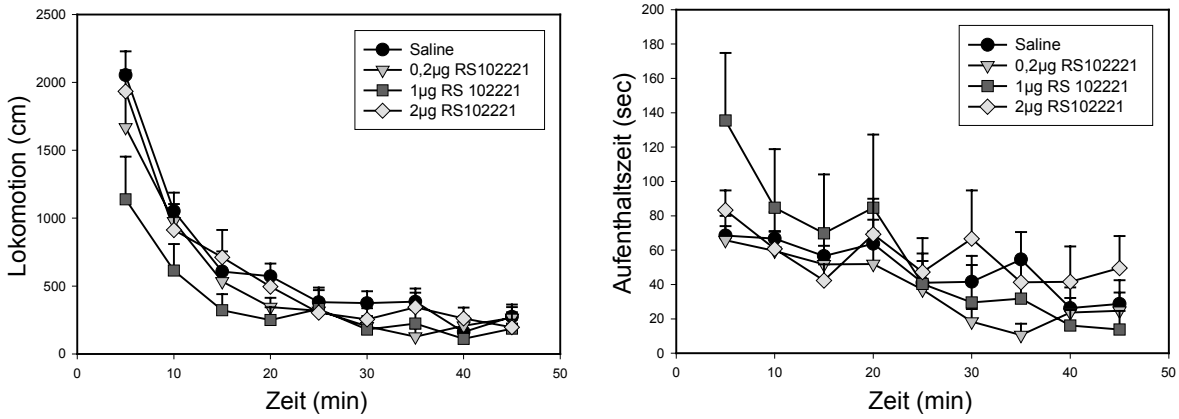

Abbildung 5: OF-Verhalten nach intrastriataler Injektion eines $5HT_{2C}$-Antagonisten Links: Lokomotion nach intrastriataler Injektion des $5HT_{2C}$-Antagonisten RS102221, MW ± SEM; Rechts: Aufenthaltszeit im Zentrum nach intrastriataler Injektion des $5HT_{2C}$-Antagonisten RS102221, MW ± SEM

Bei der automatischen USV-Aufnahme während dem OF-Test traten teilweise technische Probleme auf, so dass sich aufgrund von Datenausfällen folgende Gruppengrößen ergaben: 0,9% NaCl (n=8), 0,2µg (n=7), 1µg (n=8) und 2µg (n=9) RS102221. Alle getesteten Ratten gaben niedrigfrequente (<32kHz) USV-Rufe von sich. Die Testsituation scheint für die Tiere jedoch allgemein nicht sehr aversiv gewesen zu sein, da die meisten Tiere (89%) nur eine geringe Anzahl von Rufe abgaben (1 bis 21 Rufe / 45min). 2 Versuchstiere (Gruppen: 0,2µg und 2µg RS102221) haben deutlich mehr vokalisiert (160 bis 172 Rufe). Die meisten Rufe gab eine Ratte aus der Kontrollgruppe von sich (1467 Rufe). Aus der Literatur ist bekannt, dass eine relativ neutrale Testsituation wie ein OF oder EPM (Borta et al. 2006), im Gegensatz zu aversive Stimuli wie Fressfeinden (Blanchard et al. 1991)

oder elektrischen Reizen (van der Poel & Miczek 1991, Jelen et al. 2003, Wöhr et al. 2005, Borta et al. 2006) keine USV hervorruft.
USV-Rufe unter 32kHz werden in lange und kurze (</> 400ms) Rufe unterteilt, da nur die langen Rufe als typisch aversiv gelten (van der Poel & Miczek 1991) und die kurzen Rufe bisher nicht interpretiert werden können. Die kurzen USV-Rufe traten in dieser Studie nur vereinzelt (1 bis 13 Rufe), meist direkt am Anfang des Tests, auf. Betrachtet man die langen 22kHz-Rufe gesondert, fließen nur noch 8Tiere in die Wertung ein. Diese Tiere sind keinen bestimmten Dosisgruppen zugeordnet (Kontrolle: 2Tiere [1 und 1300 Rufe]; 0,2µg RS102221: 1Tier [131Rufe]; 1µg RS102221: 2Tiere [je 1 Ruf]; 2µg RS102221: 3Tiere [1, 20 und 152 Rufe]). Die Frequenz dieser Rufe betrug 14,1 bis 24,6kHz, die durchschnittliche Länge 400 bis 1810ms. Hierbei ist jedoch zu bedenken, dass auch die separaten Rufe nur knapp 400ms lang waren und nicht als typisch aversive USV-Rufe zu betrachten sind, da ihre Frequenz unter 18kHz lag.
Die Testsituation in diesem Versuch und auch die Injektion des $5HT_{2C}$-Antagonisten RS102221 in das ventrale Striatum führten also generell nicht zu aversiver USV.

5.3 Behavioral and neurochemical consequences of multiple MDMA administrations in the rat: role of individual differences in anxiety-related behavior

Langzeitiger MDMA Konsum ist mit psychiatrischen Erkrankungen, wie Angststörungen und Depression (Steele et al. 1994, Schifano et al. 1998), oder auch Anorexie (Vollweider et al. 1998, Parrott et al. 2002) assoziiert. Jedoch ist auch hier die Literatur inkonsistent und in Tierversuchen zeigen sich nach hoher oder wiederholter MDMA-Applikation sowohl anxiolytische (Mechan et al. 2002, Ho et al. 2004) als auch anxiogene Effekte (Morley et al. 2001, Gurtman et al. 2002). Von einigen Autoren wird vermutet, dass der Zusammenhang zwischen MDMA-Konsum und Depression vielmehr dadurch zustande kommt, dass Individuen mit mentaler Auffälligkeit anfälliger auf MDMA reagieren (Lieb et al. 2002, Huizink et al. 2006). Eine unterschiedliche Reaktionen auf MDMA ist auch zwischen Rattenlinien (Green & McGregor 2002) oder Individuen eines Rattenstammes (Ho et al. 2004) mit unterschiedlichem Angstverhalten zu finden.

In dieser Studie wurden Ratten auf ihr individuelles Angstverhalten im EPM untersucht und in LA und HA-Gruppen unterteilt, bevor sie wiederholt MDMA oder 0,9% NaCl appliziert bekamen und daraufhin im OF (im angehängten Manuskript wurde das Synonym “activity box“ verwendet), EPM und Neuen-Objekt-Test (novel object test, NO) getestet wurden. Nach den Verhaltenstests erfolgte eine neurochemische Analyse einzelner Hirnareale.

Die MDMA-Injektionen führten zu einer moderaten Verringerung des 5HT-Gehalts im ventralen Striatum. Im frontalen Kortex fand sich diesbezüglich kein Effekt. Allerdings war hier der Spiegel des 5HT-Metaboliten 5HIAA nach MDMA-Applikation bei HA-, nicht jedoch bei LA-Tieren erhöht. Im OF konnte akut der erwartete aktivierende Effekt von MDMA gezeigt werden. Nach wiederholter OF-Testung zeigte sich, in der allgemeinen Lokomotion, und speziell der Lokomotion im Zentrum, sowohl ein unterschiedlicher Effekt zwischen HA- und LA-Tieren, als auch eine Interaktion zwischen diesem individuellen Angstverhalten und der MDMA-Behandlung. Die LA-Subgruppe, bzw. die Gruppe LA-Verhalten + MDMA-Behandlung, zeigten mehr Lokomotion als die übrigen Ratten. Zwei Tage nach der wiederholten Applikation von MDMA oder 0,9% NaCl, bekamen alle Tiere MDMA verabreicht. MDMA-vorbehandelte Ratten wiesen hiernach eine höhere Lokomotion auf als NaCl-vorbehandelte Tiere, was auf eine Sensitivierung in der MDMA-Gruppe hinweist. Diese verstärkte Substanzwirkung war wiederum in den LA-Ratten deutlicher ausgeprägt als in der HA-Subgruppe. Zudem führte MDMA nur in den HA-Ratten zu einer Verringerung des Körpergewichts. Die EPM-Testung nach MDMA-Behandlung ergab hingegen nur einen Verhaltenseffekt bei den LA-Tieren. Diese zeigten im Verlauf des Tests statt der erwarteten Reduktion eine erhöhte Exploration der offenen Arme. Weiterhin führte die MDMA-Behandlung zu einer erhöhten Exploration eines neuen Objekts, ein Effekt der ebenfalls hauptsächlich auf der LA-Subgruppe beruht. Zusammenfassend zeigt sich in den behavioralen Daten, dass sich wiederholte MDMA-Applikation auf Ratten mit verschiedenem individuellen Angstverhalten differenziert auswirkt.

6. Zusammenfassende Diskussion

Die in dieser Dissertation durchgeführten Studien tragen zu einem besseren Verständnis psychologischer und neurochemischer Grundlagen der Emotion Angst und der hierzu oft inkonsistenten Literatur bei. Insgesamt sind die Mechanismen, die an Angst beteiligt sind, sehr komplex. So beeinflusst beispielsweise die individuelle Ängstlichkeit von Ratte die Reaktion auf, das auf das 5HT-System wirkende, MDMA. Zudem hat 5HT in verschieden Hirnarealen unterschiedliche Wirkung auf Angst, jedoch sind zentrale 5HT-Läsionen in den meisten Studien nicht lokal begrenzt. Injizieren die Autoren das Toxin dennoch in ein bestimmtes Hirnareal, wird oft versäumt, auch andere Bereiche und Neurotransmitter zu untersuchen.

Aus den Ergebnissen der ersten Studie, in der 5,7-DHT intrastriatal injiziert wurde, geht hervor, dass eine Reduktion von 5HT im anterioren Vorderhirn zu einer Erhöhung von Angstverhalten im EPM und OF führt. Dieses Ergebnis entspricht den Ergebnissen aus einigen anderen Untersuchungen (Schwarting el al. 1998, Otano et al. 1999, Harro et al. 2001, Gurtman et al. 2002), wobei jedoch in vielen Studien ein gegenteiliger Effekt gefunden wurde (Iversen 1984, Briley et al. 1990, Söderpalm & Engel 1992, Olausson et al. 2001). Wie bereits erwähnt, könnte dies daran liegen, dass die meisten Studien mit einer generellen Läsion von zentralem 5HT arbeiten, 5HT in verschiedenen Hirnregionen Angst jedoch genau gegenläufig moduliert (Deakin & Graeff 1991). Um die funktionale Rolle von 5HT im Zusammenhang mit Angst genau zu verstehen, ist es wichtig, die Bedeutung von 5HT in einzelnen Hirnarealen zu untersuchen. Auch in der Läsionsstudie dieser Dissertation lassen sich die gefundenen Verhaltenseffekte nicht allein auf den 5HT-Gehalt der Injektionsstelle, dem ventralen Striatum, zuordnen, da es ebenfalls zu 5HT-Läsionen im Neostriatum, dem frontalen Kortex und, zu einem geringeren Grad, in der Amygdala kam. Diese weitreichende Läsion kann dadurch erklärt werden, dass die gebündelten 5HT-Fasern, wenigen gemeinsamen Kernen entspringen, durch das ganze Vorderhirn ziehen und sich erst kurz vor den Projektionsgebieten aufteilen. Die wenigen Studien, die lokale 5HT-Läsionen erstrebt und anschließend mehrere Hirnareale analysiert haben, haben ebenfalls Läsionen abseits des Injektionsorts gefunden (Yoshimoto et al. 1995, Sommer et al. 2001). Oft untersuchen Studie jedoch keine andere Hirnreale als den Injektionsort (Chia et al. 1996, Daws et al.

1998, Chia et al. 1999, Andrade & Graeff 2001, Loskutova 2001, Anguiano-Rodríguez et al. 2007). Ein Verhaltenseffekt wird so möglicherweise auf den 5HT-Gehalt in einer bestimmten Hirnregion zurückgeführt, die jedoch nicht ausschließlich betroffen ist. Finden sich andererseits keine Effekte, bedeutet dies nicht zwangsläufig, dass 5HT in der untersuchten Hirnregion nicht an dem Verhalten beteiligt ist, da eventuell eine 5HT-Reduktion in einer anderen Hirnregion gegenteilig auf das Verhalten wirkt und Effekte so unterdrückt werden. Auch arbeiten die meisten Studien ausschließlich mit Desipramin, um catecholaminerge Transmittersysteme vor 5,7-DHT zu schützen (Björklund et al. 1975, Murtha & Pappas 1994, Rex et al. 2003). Dies reicht jedoch, zumindest bei striataler Injektion des Toxins, nicht aus, um auch DA zu schützen (Yoshimoto et al. 1995). Oft werden in 5HT-Läsionsstudien andere Neurotransmitter nicht untersucht (De Oliveira Mora et al. 1999, Hall et al. 1999, Thomas et al. 2000, Callahan et al. 2001, Netto et al. 2002, Choi et al. 2004, Beekman et al. 2005, Anguiano-Rodríguez et al. 2007), so dass die Rückschlüsse, die in diesen Studien von dem 5HT-Gehalt auf das Verhalten gezogen werden, nicht unbedingt zutreffend sind, da das Verhalten eventuell auch auf DA-Läsionen zurückzuführen sein kann.

Im aktiven Vermeidungstest der Läsionsstudie zeigten sich keine Veränderungen im beobachtbaren Verhalten; jedoch finden sich Substanzeffekte in der USV der Tiere. Die Läsion des 5HT-Systems führt zu einer Veränderung der Frequenz, also der Tonhöhe, an den Rufenden. Es ist bereits aus anderen Arbeiten bekannt, dass Frequenzunterschiede in aversiv motivierter USV eine wichtige Variable im Bezug auf Angstverhalten sind (van der Poel & Miczek 1991, Borta et al. 2006). Es empfiehlt sich demzufolge, bei aversiven Verhaltenstestungen auch die USV der Versuchstiere als zusätzliches Verhaltensmaß aufzuzeichnen, da diese eine weitere und empfindliche Verhaltenskomponente der Ratten darstellt und Substanzeffekte, die im sichtbaren Verhalten nicht auftreten, so möglicherweise aufgezeigt werden können.

In der zweiten Studie, in der ein $5HT_{2C}$-Antagonisten in das ventrale Striatum injiziert wurde, ergaben sich keine Angsteffekte im OF-Verhalten der Tiere. Es ist bekannt, dass der $5HT_{2C}$-Rezeptor an Angstverhalten beteiligt ist, jedoch wird auch hier in vielen Studien die Substanz systemisch verabreicht. Dies führt in einigen Studien zu anxiolytischen, in anderen jedoch zu anxiogenen Effekten (Scorza et al. 1996,

Kennett et al. 1997, Wood et al. 2001, Ji et al. 2006). Es ist davon auszugehen, dass der injizierte Antagonist in dieser Studie lokal am Injektionsort verbleibt, da dieser an der Membran wirkt und nicht, wie das Toxin 5,7-DHT, in die Zelle aufgenommen wird. Bei lokaler Applikation ergeben sich je nach Injektionsgebiet unterschiedliche Effekte. Overstreet et al. (2006) finden ebenfalls keine Angsteffekte nach Manipulation des $5HT_{2C}$-Rezeptors im Nacc. In der Amygdala (Campbell & Merchant 2003) und im ventralen Hippocampus (Alves et al. 2004) scheinen $5HT_{2C}$-Agonisten anxiogene Effekte zu haben, im dorsalen Hippocampus hingegen keine (Alves et al. 2004). Es läßt sich nun eventuell schlußfolgern, dass die anxiogenen Effekte in der vorangegangenen 5HT-Läsionsstudie auf andere 5HT-Rezeptortypen oder extrastriatale 5HT-Schädigungen zurückzuführen sind. Ein anderer Grund für das Fehlen von deutlichen Effekte durch intrastriatale Injektion des $5HT_{2C}$-Antagonisten RS102221 auf Angstverhalten, könnte eventuell auch darin liegen, dass diese durch Beeinflussung der Lokomotion überlagert wurden. Es ist bekannt, dass sowohl 5HT als auch das Corpus striatum mit Motoraktivität assoziiert sind (Trepel 2003, Jacobs 1991). So wirken beispielsweise niedrige und mittlere Dosen eines $5HT_{2C}$-Agonisten, in den Hippocampus injiziert, anxiogen, hohe Dosen dagegen verringern die Lokomotion (Alves et al. 2004). McMahon et al. (2001) fanden keine Lokomotionseffekte aufgrund Applikationen relativ geringer Dosen RS102221 (0,05-0,5µg) in den Nacc. Campbell & Merchant (2003) wiederum zeigten, dass sowohl ein $5HT_{2C}$-Antagonist systemisch, als auch ein $5HT_{2C}$-Agonist in die Amygdala injiziert, die Lokomotion reduzierten. Die Ergebnisse dieser Studie fügen sich insofern in die Befundlage der Literatur, da geringe Dosen des $5HT_{2C}$-Antagonisten (0,2µg) keine Lokomotionseffekte hervorrufen, höhere Dosen (1µg) hingegen schon. Da durch die intrastriatale Injektion des $5HT_{2C}$-Antagonist jedoch keine auffälligen Änderungen im Angstverhalten hervorgerufen wurden, könnte die Untersuchung eventuell mit der Manipulation anderer 5HT-Rezeptorsubtypen, wie dem $5HT_{2A}$-, $5HT_3$- oder $5HT_6$-Rezeptor, fortgeführt werden.

Die Ergebnisse der ersten Studie, in der 5HT-Läsionen gesetzt wurden, zeigen außerdem, dass Verhaltensunterschiede im OF zwischen lädierten und scheinlädierten Ratten deutlich hervortreten, wenn die Tiere zusätzlich mit MDMA behandelt werden. Diese akuten Verhaltenseffekte unter MDMA korrelieren mit dem Grad der 5HT-Schädigung durch 5,7-DHT.

In der dritten Studie, in der die Langzeiteffekte von MDMA unter der Berücksichtigung der natürlichen Ängstlichkeit der Tiere untersucht wurden, ergab sich, dass die Wirkung von wiederholter MDMA-Applikation in verschiedenen Angsttests von der vorherigen natürlichen Ängstlichkeit der Ratten abhängt. Anxiolytische Effekte durch MDMA finden sich im NO und EPM, jedoch nur bei niedrigängstlichen Tieren. Dies stimmt mit den Befunden überein, dass Stämme mit unterschiedlichem Angstverhalten sich in ihrer Reaktion auf MDMA unterscheiden (Green & McGregor 2002) und auch individuell verschieden ängstliche Tiere des gleichen Stammes auf eine einzelne hohe Dosis MDMA differenziert reagieren (Ho et al. 2004). Dass Substanzeffekte von individuellen Eigenschaften der Versuchstiere abhängen können, wurde bereits in Studien mit Tieren individuell unterschiedlicher Aktivität oder Exploration gezeigt. So sind beispielsweise hoch aktive Tiere sensitiver gegenüber DA-Agonisten (Dellu et al. 1996, Saigusa et al. 1999, Ellenbroek & Cools 2002). Zudem wirkt auch ein Muskarin-Antagonist auf Habituationseffekte im Aufrichtverhalten nur in hoch aktive Ratten (Thiel et al. 1999). Die unterschiedliche Wirkung von MDMA je nach individuellem Angstverhalten, lässt sich dadurch erklären, dass MDMA auf das 5HT-System wirkt und der intrazerebrale 5HT-Gehalt bei hoch und niedrig ängstlichen Tieren wiederum verschieden ausfällt (Schwarting et al. 1998). Die von den individuellen Eigenschaften des Versuchstieres abhängige Wirkung von MDMA kann die uneinheitlichen Ergebnisse in der Literatur, die mit neurotoxischen Dosen von MDMA sowohl anxiolytische (Mechan et al. 2002, Ho et al. 2004), anxiogene (Morley et al. 2001, Gurtman et al. 2002) oder keine Effekte finden (Bull et al. 2004, Ho et al. 2004, Sumnall et al. 2004), teilweise erklären. Da nicht alle Stämme oder Individuen eines Stammes auf eine Manipulation des 5HT-Systems gleich reagieren (Bert et al. 2001), sollten Versuchstiere vorher auf ihre natürliche Ängstlichkeit untersucht werden, da sonst möglicherweise Effekte, die deutlich, aber nur in einer Subgruppe auftreten, verschwinden können.

Insgesamt sind die zerebralen Mechanismen, die an Angstverhalten beteiligt sind, sehr komplex. Um das neuronale Angstsystem und seine einzelnen Abschnitte genauer zu verstehen und Angsterkrankungen gezielter behandeln zu können, müssen Neurotransmitter und Hirnstrukturen im Einzelnen auf ihren Zusammenhang zu Angst untersucht werden. Hierbei ist es jedoch wichtig, auch die nicht gezielt manipulierten Neurotransmitter und Hirnstrukturen zu analysieren, um

sicherzugehen, dass eine Beeinflussung des Nervensystems nicht umfangreicher als erwartet ist und falsche Schlussfolgerungen in dem Zusammenhang von Neurochemie und Verhalten gezogen werden. Zudem sind die individuellen Verhaltenseigenschaften eines Lebewesens durch seine differentielle Neurochemie beeinflusst, die sich wiederum auf die Effekte auswirkt, die durch Manipulationen des 5HT-Systems herbeigeführt werden. Versuchstiere sollten demnach auch vor einer pharmakologischen Behandlung getestet werden.

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Hiermit erkläre ich, die vorliegende Arbeit selbständig und nur unter der Verwendung der angegebenen Quellen und Hilfsmittel verfasst zu haben.
Diese Dissertation wurde in der jetzigen oder einer ähnlichen Form bei keiner anderen Hochschule eingereicht.

Marburg, den 30.05.2007

(Dipl.-Biol. Verena Ludwig)

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Neurochemical and behavioral consequences of striatal injection of 5,7-dihydroxytryptamine

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Abstract

It is known that central serotonin (5HT) is involved in anxiety, but the behavioral results of many studies have been inconsistent. A prevalent research approach is to destroy 5HT neurotoxically. Such lesions were mostly generated by injecting 5,7-dihydroxytryptamine into ventricles or raphé nuclei, leading to rather global losses of 5HT in the brain. However, there is evidence for differential effects of 5HT in different brain structures regarding anxiety. Therefore, we decided to study the effects of injecting 5,7-dihydroxytryptamine into the forebrain. We chose the ventral striatum as the site of injection, since there is evidence that 5HT may be involved in anxiety there. We administered the neurotoxin bilaterally in adult rats, and analyzed neurochemical and behavioral consequences in three experiments. The first one showed that the toxin dose-dependently (10–50 μg) depleted 5HT in the ventral striatum, neostriatum, frontal cortex, and amygdala. Besides 5HT, dopamine was also partly depleted there. This dopaminergic lesion was prevented in a second experiment, where rats were pre-treated systemically with the dopamine reuptake inhibitor nomifensine. In the final experiment, the functional consequences of such 5HT lesions were tested, which yielded moderate anxiogenic effects in the elevated plus maze and in the open field. Also, there were lesion effects on aversively motivated ultrasonic vocalization during an active avoidance test. In contrast, active avoidance performance itself and general activity in the open field were not affected. Lesion effects became discernible there when challenging rats with MDMA. The psycho-stimulatory effectiveness of this drug, which acts largely via the availability of 5HT in the brain, was reduced to degrees that depended on the size of 5HT lesion. These results are discussed with respect to factors such as severity of lesion, anatomical specificity, and the role of 5HT in anxiety.

Keywords: Serotonin; Dopamine; 5,7-Dihydroxytryptamine; Nomifensine; MDMA; Ventral striatum; Neostriatum; Frontal cortex; Amygdala; Elevated plus maze; Anxiety; Avoidance; Locomotion; Ultrasonic vocalization

1. Introduction

Serotonin (5-hydroxytryptamine, 5HT) in the brain seems to have important modulatory effects on emotion, since it is known that central 5HT is involved in anxiety (Handley and McBlane, 1993; Lowry et al., 2005). The classic hypothesis states that a general decrease of 5HT leads to an anxiolytic effect (Briley et al., 1990; Iverson, 1984). One major research approach is to study the effects of destroying 5HT neurons in the brain. Mostly, rather general 5HT losses were established by injecting a toxin into ventricles (e.g. Briley et al., 1990; Hall et al., 1999), or into the raphé nuclei (e.g. Andrade and Graeff, 2001; Rex et al., 2003). In contrast, there are only a few studies, in which the toxin was administered locally into specific 5HT projection sites (Bland et al., 2004; Chia et al., 1999; Sommer et al., 2001). It is known, however, that the role of 5HT in anxiety may differ critically between certain brain sites receiving 5HT innervation, like hippocampus, amygdala, striatum, prefrontal cortex, and periaqueductal gray (Fendt and Fanselow, 1999; File et al., 1996; Rex et al., 1993). Even more, Deakin and Graeff (1991) proposed a dual role of 5HT with anxiogenic properties in the amygdala, in contrast to anxiolytic ones in the periaqueductal gray. Such intricate brain mechanisms may be one of the reasons, why behavioral results with general 5HT lesions have been inconsistent, and why the role of local 5HT transmission in anxiety-related behavior is still not well understood.

Here, we wanted to address this issue by studying neurochemical and behavioral effects of neurotoxic injections into 5HT projection sites. As a target, we chose the ventral striatum, with the nucleus accumbens as its main component. This brain area is thought to constitute an interface between limbic inputs and motor output, and may serve as an important structure for the

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transfer from motivation into behavior (Spoont, 1992). Among others, it receives 5HT innervation from the dorsal and median raphé nuclei. Schwarting et al. (1998) showed that the ventral striatum is supposedly involved in anxiety, since they found that rats with different individual levels of anxiety-related behavior, as tested in the elevated plus maze, differed regarding their 5HT tissue levels in the ventral striatum. Such rats also differed in active avoidance behavior (Ho et al., 2002) and in aversively motivated ultrasonic vocalization (Borta et al., 2006). Otano et al. (1999) also addressed the possible relevance of 5HT in the nucleus accumbens regarding anxiety, and found that reducing $5HT_6$ receptor levels there led to enhanced anxiety-related behavior, for example in the elevated plus maze.

To destroy 5HT, we selected the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT; Baumgarten and Lachenmayer, 2004), which is the most prevalent method to lesion central 5HT (e.g. Briley et al., 1990; Thomas et al., 2000). In a series of experiments, we first varied its dose to find an optimal neurotoxic concentration. Then, we additionally gave different doses of a dopamine reuptake inhibitor to study protection of dopaminergic neurons. Finally, we tested the effect of such 5HT lesions on A) anxiety-related behavior in the elevated plus maze, B) active avoidance and simultaneous ultrasonic vocalization in the shuttle box, and C) activity in the open field. There, we also tested the stimulatory effects of 3,4-methylenedioxymethamphetamine (MDMA), which is known to act largely via 5HT systems in the brain (for review see Cole and Sumnall, 2003).

2. Methods

2.1. Animals

Adult male Wistar rats (Harlan Winkelmann, Germany) weighing 215–273 g at the time of arrival were used. They were housed in groups of 3–4 rats in acrylic cages (35 cm × 56 cm × 34 cm) with food and water available *ad libitum* and a 12/12 h light/dark cycle (lights on at 07:00 h). Each animal was handled on several consecutive days (5 min each day) prior to surgery and behavioral testing.

2.2. Surgery

In the first experiment, the dose of the neurotoxin (5,7-DHT creatinine sulfate, Sigma, Germany) was varied systematically. All animals received the reuptake inhibitor desipramine (25 mg/kg desipramine hydrochloride i.p., Sigma, Germany) 30 min before surgery in order to protect noradrenergic neurons from the toxin (Björklund et al., 1975; Choi et al., 2004). Under anesthesia with ketaminhydrochloride (0.45 ml/kg Ketavet, Pharmacia, Germany) and xylazin (0.2 ml/kg Rompun, Bayer, Germany), they were placed in a stereotaxic frame (TSE Systems, Germany). The neurotoxin was dissolved to concentrations of 10 ($n = 7$), 25 ($n = 7$), or 50 μg ($n = 7$), which is equivalent to 4.8, 11.9, or 23.8 μg of the free base, respectively. The solvent (saline; 0.9% NaCl containing 0.1% ascorbic acid) was administered in the control group ($n = 6$). Injections were made bilaterally, each with a volume of 2 μl (0.35 μl/min; guide cannula 26 gauge, internal cannula 33 gauge, Plastics One, USA). The coordinates for infusion sites were 1.6 mm anterior and ±1.8 mm lateral from bregma and 7.4 mm ventral from skull surface (Paxinos and Watson, 1997). The incisor bar was set at 3.3 mm below interaural line. The cannula was left *in situ* for 5 min after the infusion was completed.

In the second experiment, we applied 25 mg/kg desipramine and either 15 or 25 mg/kg i.p. of the dopaminergic reuptake inhibitor nomifensine (nomifensine maleate salt, Sigma, Germany) to also protect dopaminergic neurons from 5,7-DHT (25 μg). Since mortality was rather high in a pilot study to this experiment, which we attributed to interactions between reuptake inhibitors and anesthesia, we moved to the anesthetic Avertin (1 ml/100 g: 1 g 2,2,2-tribromoethanol in 0.5 g tertiary amyl alcohol, 4 ml ethanol and 45 ml phosphate-buffered saline; Fluka, Switzerland). We also used different cannula sizes (guide cannula 23 gauge, internal cannula 30 gauge), a smaller volume of injection (1 μl/site), and a slower velocity of injection (0.22 μl/min). The following group sizes were used: saline + 15 mg/kg nomifensine ($n = 6$), toxin + 15 mg/kg nomifensine ($n = 6$), saline + 25 mg/kg nomifensine ($n = 5$), and toxin + 25 mg/kg nomifensine ($n = 4$).

In the third experiment, where behavioral consequences were also tested, we used 25 mg/kg desipramine, 15 mg/kg nomifensine and 25 μg/1 μl 5,7-DHT or saline (saline $n = 8$, toxin $n = 13$). The surgical procedure was otherwise the same as in the second experiment.

2.3. Behavioral tests

Behavioral tests in experiment 3 were started 9 days after surgery. First, the rats were tested in the elevated plus maze, a pharmacologically validated and well-established animal model of anxiety (Rodgers et al., 1997). Three days later, active avoidance performance was analyzed. Here, ultrasonic vocalization was also measured. After another 3-day break, rats were tested in an open field. In this test, they were additionally treated with MDMA (5 mg/kg s.c., Lipomed, Switzerland) dissolved in 1 ml/kg saline. All behavioral tests were conducted during the light phase of diurnal rhythm.

2.3.1. Elevated plus maze (EPM)

The EPM consisted of two opposed open arms (50 cm × 10 cm), two opposed enclosed arms with no roof (50 cm × 10 cm × 40 cm), and an open square (10 cm × 10 cm) in the center. The open arms were surrounded by a small rim (4 mm × 8 mm). The maze was elevated 50 cm above the floor and was monitored by a video camera from above. Testing was conducted under white light (30 lx in the center), and was started by placing the rat into the center of the maze, facing one of the open arms. Each rat was tested on two consecutive days (day 1: 5 min, day 2: 15 min). Latencies to enter open (distal or proximal) or enclosed arms and number of entries into and time spent on the respective arms were analyzed from videotapes. An entry into any of the compartments was defined as all four paws being placed there.

2.3.2. Two-way active avoidance

A two-way shuttle box (33 cm × 66 cm × 39 cm) was illuminated with white light (30 lx in the center) and monitored by a video camera from above. The floor was made of 2 mm diameter steel rods spaced 1.5 cm apart. The box was divided into two equal compartments by a barrier (height: 5 cm, width: 0.6 cm). Each compartment could be electrified separately through a shock scrambler (ENV-141S, Med Associates, England). A speaker was mounted in the center on the top of the box. The animals were placed into the shuttle box and allowed to explore the entire apparatus for 2 min. Then, they received 20 shuttle trials. Each trial started with an 80 dB tone (frequency 1.9–14.6 kHz), which lasted 3 s, followed by a 0.3 mA scrambled foot shock. If the animal crossed the barrier during shock delivery, an escape response was measured. If it failed to cross, the shock was terminated after 15 s (failed escape). If the animal crossed the barrier during the tone, no shock was delivered and an avoidance response was counted. After 45 s, the next trail was initiated. The latencies to avoid or escape, and the numbers of avoidances, escapes and failed escapes were analyzed from videotapes.

2.3.3. Ultrasonic vocalization (USV)

During active avoidance, rat USV was also measured (for details see Wöhr et al., 2005). Here, the following modified settings were used: sampling rate 250 kHz, spectrogram generation with a time window overlap of 75% (Hamming window), automatic threshold-based algorithm (threshold: −55 dB) and hold-time mechanism (hold-time: 10 ms) for call detection, post filters on 20 ms for minimal call duration and 0.3 for maximal entropy. Based on their lengths, calls were divided into short and long ones (</>400 ms), since long calls are considered as typical aversive calls (van der Poel and Miczek, 1991).

2.3.4. Open field (OF)

The OF used consisted of a box (41 cm × 41 cm × 40 cm) which was monitored by an automated activity monitoring system (TruScan, Photo beam Sensor-E63-22, Coulbourn Instruments, USA). Behavior was tested under red light (28 lx in the center) with three treatment conditions. First, the rats were allowed to explore the novel OF for 30 min, then, they were tested for 30 min after an injection (s.c.) of saline, followed by a 60 min test after an injection (s.c.) of MDMA. Locomotion, center time and entries (defined as the animal's center of gravity being within the 20 cm × 20 cm center area of the OF), and the number of rearings were measured.

2.4. Neurochemical analysis

The animals were lightly anaesthetized with Narkoren (0.3 ml, Merial, Germany) and decapitated. The brains were quickly removed and the frontal cortex, ventral striatum, neostriatum, and amygdala were dissected out bilaterally, homogenized in 0.05 M perchloric acid, and stored at −80 °C. The samples were analyzed for their contents of 5HT, 5-hydroxyindole acetic acid (5HIAA), dopamine (DA), and dihydroxyphenylacetic acid (DOPAC) using high-performance liquid chromatography (HPLC) with electrochemical detection (Antec Leyden BV, The Netherlands). The biogenic amines were separated on a Nucleosil 100-5 C18 column (125 mm × 4 mm, particle size 5 μm, Macherey-Nagel, Germany) using a mobile phase containing 35 ml/l acetonitrile, 140 mg/l octanesulphonic acid, 100 mg/l Na_2EDTA, and 6 ml/l triethylamine (pH 2.95). The detector potential was set at 600 mV relative to an Ag/AgCl reference electrode.

2.5. Statistical analysis

All results were expressed as means ± S.E.M. Statistical testing was performed using either *t*-tests or analyses of variance (ANOVA), followed by Scheffé tests. Furthermore, Pearson's correlation coefficient was used to compare behavior with neurochemical data. All *p*-values are two-tailed and taken as significant when below 0.05.

3. Results

3.1. Experiment 1: role of toxin dose

In the lesion groups, 5HT and 5HIAA were significantly decreased in the ventral striatum (p-values <0.001; Table 1). The mean level of 5HT reduction was 62% in the 10 μg toxin group, 78% in the 25 μg group, and 86% in the 50 μg group as compared to controls. Furthermore, 5HT depletion was significantly stronger in the 50 μg toxin group than in the 10 μg group ($p = 0.020$). Compared to controls, 5HIAA was also significantly reduced in the 10 μg (57%), 25 μg (72%), and 50 μg group (78%, all p-values <0.001).

Apart from the ventral striatum, the toxin also depleted 5HT in the neostriatum, the frontal cortex, and the amygdala; similar results were obtained with respect to 5HIAA (all p-values <0.001). The reduction of 5HT in the neostriatum was 63% in the 10 μg toxin group, 68% in the 25 μg group, and 81% in the 50 μg group as compared to the control group. In the frontal cortex, the mean level of 5HT reduction was 69% in the 10 μg toxin group, 81% in the 25 μg group, and 85% in the 50 μg group. In the amygdala, 5HT reduction was 32% in the 10 μg toxin group, 39% in the 25 μg group, and 65% in the 50 μg group. 5HT depletion here was significantly stronger in the 50 μg toxin group than in the 10 μg ($p = 0.003$) or 25 μg group ($p = 0.023$).

Furthermore, 5,7-DHT led to reductions of DA in the ventral striatum, where DA was reduced by 32% ($p = 0.045$) in the 25 μg toxin group and by 49% ($p = 0.001$) in the 50 μg group. In the neostriatum, there were only trends for DA decreases (10 μg: $p = 0.086$, 25 μg: $p = 0.250$, 50 μg: $p = 0.053$). In the frontal cortex ($p = 0.975$), and the amygdala ($p = 0.835$), DA levels were not reduced. In the ventral striatum, DOPAC was also significantly decreased in the 25 μg (48%, $p = 0.007$) and 50 μg (78%, $p = 0.001$) toxin groups, but not the 10 μg group ($p = 0.151$).

3.2. Experiment 2: effects of DA reuptake inhibitor

Due to the significant reductions of DA and DOPAC in the first experiment, we additionally applied the DA reuptake inhibitor nomifensine to prevent animals from such damage.

Table 1
Dose–response effects of intrastriatal 5,7-DHT

Region	Treatment	5HT	5HIAA	DA	DOPAC
Ventral striatum	Control	1.198 ± 0.048	0.573 ± 0.078	5.994 ± 0.704	1.632 ± 0.254
	10 μg 5,7-DHT	0.450 ± 0.095$^{**,\#}$	0.246 ± 0.040**	5.002 ± 0.320$^{\#}$	1.146 ± 0.064
	25 μg 5,7-DHT	0.268 ± 0.045**	0.161 ± 0.014**	4.066 ± 0.185*	0.841 ± 0.065*
	50 μg 5,7-DHT	0.165 ± 0.019**	0.128 ± 0.077**	3.035 ± 0.455**	0.664 ± 0.131**
Neostriatum	Control	0.479 ± 0.022	0.483 ± 0.033	14.532 ± 0.682	1.638 ± 0.078
	10 μg 5,7-DHT	0.177 ± 0.025**	0.209 ± 0.029**	11.760 ± 0.660	1.256 ± 0.089
	25 μg 5,7-DHT	0.152 ± 0.022**	0.173 ± 0.017**	12.405 ± 0.792	1.169 ± 0.093
	50 μg 5,7-DHT	0.093 ± 0.015**	0.128 ± 0.013**	11.507 ± 0.652	1.391 ± 0.231
Frontal cortex	Control	0.575 ± 0.029	0.238 ± 0.010	0.114 ± 0.023	0.098 ± 0.009
	10 μg 5,7-DHT	0.178 ± 0.033**	0.092 ± 0.013$^{**,\#}$	0.132 ± 0.071	0.075 ± 0.009
	25 μg 5,7-DHT	0.112 ± 0.011**	0.062 ± 0.003**	0.108 ± 0.031	0.093 ± 0.015
	50 μg 5,7-DHT	0.085 ± 0.013**	0.050 ± 0.008**	0.135 ± 0.058	0.063 ± 0.008
Amygdala	Control	0.780 ± 0.036	0.350 ± 0.028	0.876 ± 0.061	0.113 ± 0.008
	10 μg 5,7-DHT	0.531 ± 0.047$^{*,\#}$	0.252 ± 0.027$^{*,\#}$	0.756 ± 0.123	0.118 ± 0.015
	25 μg 5,7-DHT	0.476 ± 0.054$^{**,\#}$	0.199 ± 0.016**	0.819 ± 0.076	0.109 ± 0.011
	50 μg 5,7-DHT	0.271 ± 0.030**	0.143 ± 0.016**	0.775 ± 0.107	0.098 ± 0.010

Values reflect brain tissue levels in μg/g (means ± S.E.M.). $^{*}p \leq 0.05$ compared with control; $^{**}p \leq 0.001$ compared with control; $^{\#}p \leq 0.05$ compared with 50 μg 5,7-DHT.

The results (Table 2) show that both doses of nomifensine were effective to prevent DA damage, since the ANOVAs did not indicate group differences of DA or DOPAC in the ventral striatum, frontal cortex, or the amygdala (p-values between 0.200 and 0.934).

On the other hand, 5HT was still significantly decreased in the ventral striatum, namely by 61% in the group with 15 mg/kg nomifensine ($p < 0.001$) and by 47% in the group with 25 mg/kg nomifensine ($p = 0.004$; compared to respective saline controls). There was no significant reduction of 5HIAA ($p = 0.073$). Again, 5HT was also depleted outside the ventral striatum, namely in the neostriatum (51%), frontal cortex (75%), and amygdala (20%, all p-values <0.01). 5HIAA was decreased in the frontal cortex ($p < 0.001$) and the amygdala ($p = 0.003$).

3.3. Experiment 3: behavioral effects

3.3.1. Elevated plus maze

In the first EPM test, which lasted 5 min, the percentages of time spent in the open arms or numbers of open arm entries did not differ between lesion and control group (p-values >0.100; Table 3). It should be noted, however, that one rat in the lesion group spent an exceptional amount of time in the open arms (Fig. 1). When excluding this outlier, open arm time differed

Table 2
Protective effects of nomifensine

Region	Treatment	Nomifensine (mg/kg)	5HT	5HIAA	DA	DOPAC
Ventral striatum	Control	15	0.976 ± 0.024	0.361 ± 0.035	5.821 ± 0.215	0.966 ± 0.036
		25	0.934 ± 0.042	0.316 ± 0.038	5.501 ± 0.388	0.955 ± 0.071
	Lesion	15	0.378 ± 0.064$^{**,\#\#}$	0.247 ± 0.020	5.124 ± 0.264	0.863 ± 0.047
		25	0.498 ± 0.128$^{**,\#}$	0.255 ± 0.042	4.811 ± 0.508	0.898 ± 0.064
Neostriatum	Control	15	0.339 ± 0.015	0.326 ± 0.043	n.d.	1.005 ± 0.066
		25	0.356 ± 0.034	0.269 ± 0.025	n.d.	1.033 ± 0.083
	Lesion	15	0.164 ± 0.018$^{**,\#\#}$	0.167 ± 0.014	n.d.	1.074 ± 0.095
		25	0.183 ± 0.023$^{*,\#\#}$	0.366 ± 0.178	n.d.	1.030 ± 0.063
Frontal cortex	Control	15	0.447 ± 0.036	0.194 ± 0.019	0.073 ± 0.019	0.081 ± 0.012
		25	0.428 ± 0.030	0.194 ± 0.011	0.180 ± 0.072	0.078 ± 0.007
	Lesion	15	0.121 ± 0.022$^{**,\#\#}$	0.063 ± 0.004$^{**,\#\#}$	0.146 ± 0.033	0.092 ± 0.007
		25	0.104 ± 0.009$^{**,\#\#}$	0.068 ± 0.008$^{**,\#\#}$	0.131 ± 0.028	0.081 ± 0.009
Amygdala	Control	15	0.857 ± 0.015	0.433 ± 0.017	0.851 ± 0.082	0.153 ± 0.015
		25	0.804 ± 0.037	0.424 ± 0.024	0.883 ± 0.078	0.155 ± 0.014
	Lesion	15	0.685 ± 0.047*	0.336 ± 0.012**	0.764 ± 0.040	0.142 ± 0.019
		25	0.643 ± 0.047*	0.364 ± 0.023	0.803 ± 0.104	0.161 ± 0.013

Values reflect brain tissue levels in μg/g (means ± S.E.M.). n.d., not detected because of technical problems. $^{*}p \leq 0.05$ compared with control + 15 mg nomifensine; $^{**}p \leq 0.001$ compared with control + 15 mg nomifensine; $^{\#}p \leq 0.05$ compared with control + 25 mg nomifensine; $^{\#\#}p \leq 0.001$ compared with control + 25 mg nomifensine.

Table 3
Lesion effects on plus maze behavior

Treatment	EPM1	EPM2		
	1–5 min	1–5 min	6–10 min	11–15 min
Open arm time (%)				
Control	26.45 ± 5.00	10.09 ± 4.09	8.96 ± 3.13	2.87 ± 1.03
Lesion	14.75 ± 4.98	9.24 ± 2.34	10.11 ± 3.07	8.31 ± 3.43
Open arm latency (s)				
Control	13.27 ± 7.68	307.03 ± 125.49		
Lesion	52.73 ± 22.94	256.21 ± 88.28		
Distal open arm latency (s)				
Control	66.27 ± 35.60	468.68 ± 136.65		
Lesion	187.70 ± 39.34*	408.20 ± 113.52		
Open arm entries (*n*)				
Control	7.38 ± 1.32	2.13 ± 0.99	2.50 ± 0.80	0.38 ± 0.18
Lesion	4.38 ± 1.23	3.15 ± 0.72	2.54 ± 0.74	1.23 ± 0.46
Total arm entries (*n*)				
Control	14.75 ± 1.63	8.13 ± 1.72	5.75 ± 1.08	3.88 ± 1.09
Lesion	9.92 ± 1.79	8.69 ± 1.15	6.08 ± 1.02	3.69 ± 0.85

Values reflect means ± S.E.M. * $p \leq 0.05$ compared with control.

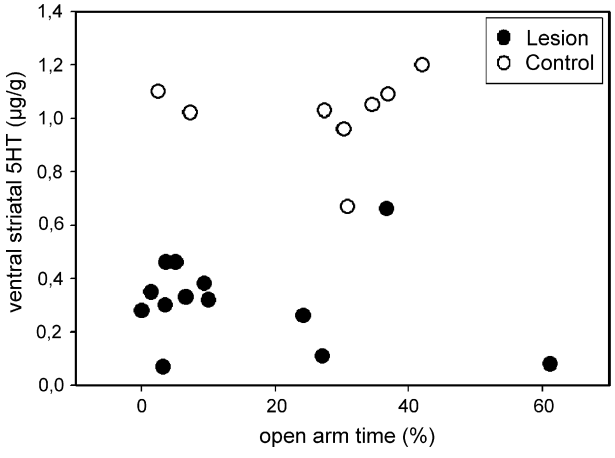

Fig. 1. Scatter-plot depicting individual relationships between open arm time (% of total arm time) in the elevated plus maze and 5HT levels in the ventral striatum (µg/g brain tissue). Filled symbols reflect animals with 5,7-DHT lesions ($n = 13$) and open symbols reflect controls ($n = 8$).

significantly between groups ($p = 0.015$), that is, rats with lesions spent less time there. Since we could not detect any cause for this outlier, we did not exclude its data in general. The latencies until the first visit of the distal part of the open arms ($p = 0.034$) were higher in the lesion group, whereas there was no significant difference in case of the proximal part ($p = 0.206$). Analyzing the number of total arm entries as a measure of activity showed a trend for less arm entries in the lesion group ($p = 0.082$).

The second EPM test was performed on the subsequent day and lasted 15 min. Here, behavior was analyzed in three time blocks of 5 min each. When comparing these between groups, we did not obtain any treatment differences (lesion, control) nor interactions between treatments and time blocks (*p*-values >0.05).

When comparing EPM behavior between both test days (0–5 min), the typical experience-dependent decrease in open arm time during the second EPM test was observed in the control group ($p = 0.010$), whereas such a decrease was not found in the lesion group ($p = 0.330$). In contrast, open arm latencies increased in both groups (control: $p = 0.046$; lesion: $p = 0.027$) and number of open arm entries decreased in both groups (control: $p = 0.002$; lesion: $p = 0.039$) on the second test day. Furthermore, the number of total arm entries (control: $p = 0.006$; lesion: $p = 0.448$) decreased only in control rats on day 2.

3.3.2. Two-way active avoidance

One rat from the lesion group had to be excluded from the analysis, since it managed to jump and remain on the barrier separating the two compartments of the testing apparatus, instead of shuttling between them. In the remaining rats (Table 4), the latencies to shuttle into the safe compartment and numbers of

Table 4
Active avoidance performance

Treatment	Response latencies (s)	Avoidances (*n*)	Escapes (*n*)	Failed escapes (*n*)
Control	4.89 ± 1.35	8.25 ± 1.35	10.00 ± 1.07	1.75 ± 1.61
Lesion	4.32 ± 0.76	8.00 ± 0.98	10.92 ± 0.73	1.08 ± 0.83

Values reflect means ± S.E.M.

Table 5
Analysis of call parameters during active avoidance behavior

	USV type	Treatment	
Call rate during tone presentation (calls/min)	All 22 kHz types	Control	5.00 ± 4.72[#]
		Lesion	0.46 ± 0.31[#]
Call rate during ISI (approx. calls/min)	All 22 kHz types	Control	16.47 ± 5.72
		Lesion	18.57 ± 5.21
Call rate (number of)	Short 22 kHz	Control	8.75 ± 3.28
		Lesion	6.77 ± 2.01
Total time spent calling (sec)	Short 22 kHz	Control	2.37 ± 0.97
		Lesion	1.66 ± 0.57
Mean peak frequency (kHz)	Short 22 kHz	Control	24.40 ± 0.59
		Lesion	24.73 ± 0.53
Call rate (number of)	Long 22 kHz	Control	238.25 ± 82.97
		Lesion	271.77 ± 76.34
Total time spent calling (sec)	Long 22 kHz	Control	217.92 ± 73.34
		Lesion	260.66 ± 64.94
Mean peak frequency (kHz)	Long 22 kHz	Control	23.64 ± 0.49
		Lesion	24.09 ± 0.40
Maximum frequency at call start (kHz)	Long 22 kHz	Control	31.29 ± 1.08
		Lesion	33.15 ± 1.22
Maximum frequency at call end (kHz)	Long 22 kHz	Control	31.64 ± 0.14
		Lesion	29.55 ± 0.70[*]
Frequency bandwidth at call start (kHz)	Long 22 kHz	Control	4.53 ± 0.92
		Lesion	7.20 ± 1.69
Frequency bandwidth at call end (kHz)	Long 22 kHz	Control	6.71 ± 0.63
		Lesion	4.72 ± 0.52[*]

Values reflect means ± S.E.M. ISI = inter-stimulus interval.
[#] $p \leq 0.05$ compared with inter stimulus interval; [*] $p \leq 0.05$ compared with control.

avoidances, escapes or failed escapes (all p-values >0.100) did not differ between lesion and controls.

3.3.3. Ultrasonic vocalization

During the active avoidance test, frequent USV was detected except for three rats with lesions and two control rats. Calls with a mean peak frequency between 32 and 51 kHz were detected in only 3 rats (lesion group, between 1–3 calls in total). These calls were excluded from further analysis, since they are not considered as aversive calls (Knutson et al., 2002). The vast majority of calls were emitted below 32 kHz, i.e. they were presumably aversive calls. These were only rarely detected during tone presentation, but occurred especially during the inter-stimulus-intervals (Table 5). Their call rates did not differ between groups (CS: $p = 0.369$; ISI: $p = 0.766$).

These calls below 32 kHz could be differentiated into short and long ones. The minority of them (3%) belonged to the short class, which ranged around 230 ms in duration, and which did not differ between groups with respect to call rate, total time spent calling, or mean peak frequency (p-values >0.05). The majority of calls (97%) were of long duration, which ranged around 1000 ms. These calls also did not differ between groups with respect to call rate, total time spent calling, or mean peak frequency (p-values >0.05). However, when differentiating between frequencies at the start versus the end of calls, it was found that rats with lesions had a lower maximum frequency at the call endpoint than control rats ($p = 0.015$). Furthermore, frequency bandwidth at the end of calls was also lower in animals with lesions ($p = 0.032$).

3.3.4. Open field

Neither in the novel OF, nor after the subsequent injection of saline, there were any differences in rearing, locomotion, or center activity between lesion and control rats (Fig. 2). MDMA treatment led to enhanced activity, but this effect was less expressed in rats with lesions (locomotion: $p = 0.001$; rearing: $p = 0.005$; center entries: $p = 0.019$; center time: $p = 0.004$).

3.3.5. Neurochemistry

Similar to experiments 1 and 2, 5HT and 5HIAA were significantly decreased (Table 6) in the ventral striatum (5HT: 69%, 5HIAA: 59%), neostriatum (5HT: 67%, 5HIAA: 57%), frontal cortex (5HT: 80%, 5HIAA: 71%), and amygdala (5HT: 29%, 5HIAA: 26%) of rats with lesions (all p-values <0.001). Similar to experiment 2, there was no reduction of DA or DOPAC in the ventral striatum, neostriatum, frontal cortex, or amygdala (all p-values >0.05).

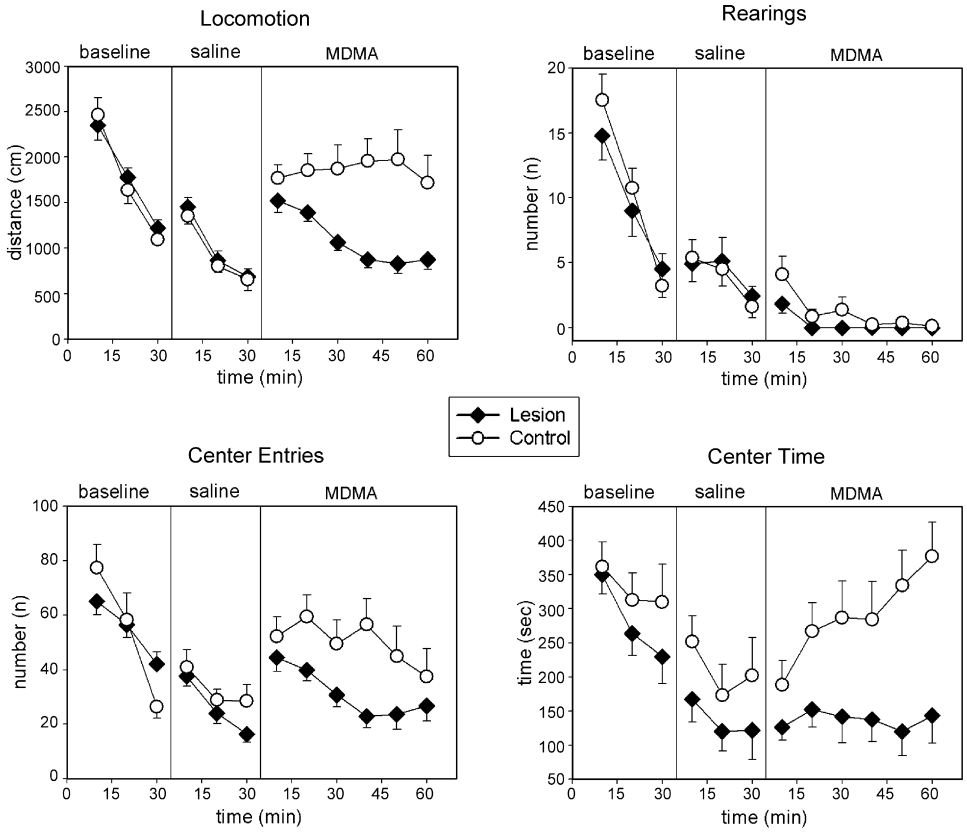

Fig. 2. Open field behavior during three consecutive tests, expressed in blocks of 10 min each. During the first block (left parts of each graph), the rats were tested for 30 min without any treatment. Then (middle parts), they were tested for another 30 min after an s.c. injection of saline. Finally (right parts), they were tested for 60 min after an s.c. injection of MDMA (5 mg/kg). Behavior was scored as locomotion (distance traveled in cm, upper left), rearing (numbers of, upper right), center entries (numbers of, lower left), and times spent in the center (in s, lower right). Filled symbols (means ± S.E.M.) reflect animals with 5,7-DHT lesions ($n = 13$) and open symbols reflect controls ($n = 8$).

3.3.6. Relations between neurochemistry and behavior

In the novel OF, center time was correlated with residual 5HT in the frontal cortex ($r = 0.601$, $p = 0.030$) and locomotion in the center was correlated with residual 5HT in the ventral striatum ($r = 0.561$, $p = 0.046$) and neostriatum ($r = 0.608$, $p = 0.027$). After the subsequent saline injection, center time was no longer correlated with 5HT levels in the lesion group, whereas in controls, it was correlated with 5HT in the amygdala ($r = 0.790$, $p = 0.020$). Rearing and locomotion in the novel OF, or after saline injection, were not correlated with 5HT levels in any brain structure.

In contrast, the loss of 5HT was correlated with the subsequent effects of MDMA, since its stimulatory effectiveness (calculated as the area under the curve, Fig. 2) decreased with more substantial 5HT depletions. Thus, in the ventral striatum, residual 5HT was correlated with locomotion ($r = 0.717$, $p = 0.0.006$) and center entries ($r = 0.797$, $p = 0.001$). Similar patterns were observed in case of the neostriatum and the amygdala (p-values between 0.002 and 0.011). In contrast, there were no substantial correlations between residual 5HT in the frontal cortex and behavior after MDMA treatment (p-values between 0.141 and 0.796). In control animals, there were no such corre-

Table 6
Lesion effects on neurochemistry

Region	Treatment	5HT	5HIAA	DA	DOPAC
Ventral striatum	Control	1.015 ± 0.055	0.408 ± 0.025	6.117 ± 0.216	0.873 ± 0.025
	Lesion	0.314 ± 0.046**	0.169 ± 0.018**	5.802 ± 0.180	0.930 ± 0.062
Neostriatum	Control	0.356 ± 0.019	0.275 ± 0.020	11.499 ± 0.524	1.031 ± 0.046
	Lesion	0.119 ± 0.012**	0.118 ± 0.011**	11.444 ± 0.422	1.064 ± 0.028
Frontal cortex	Control	0.475 ± 0.025	0.168 ± 0.009	0.121 ± 0.036	0.046 ± 0.004
	Lesion	0.097 ± 0.007**	0.049 ± 0.002**	0.085 ± 0.010	0.046 ± 0.002
Amygdala	Control	0.902 ± 0.031	0.331 ± 0.014	0.726 ± 0.078	0.133 ± 0.007
	Lesion	0.644 ± 0.038**	0.246 ± 0.015**	0.847 ± 0.040	0.157 ± 0.008

Values reflect brain tissue levels in μg/g (means ± S.E.M). ** $p \leq 0.001$ compared with control.

lations (p-values between 0.167 and 0.907), except for a trend between neostriatal 5HT and center time ($r = -0.682$, $p = 0.062$).

4. Discussion

This study shows that administration of 5,7-DHT into the ventral striatum led to a dose-dependent depletion of 5HT there; however, substantial 5HT lesions were also detected in neostriatum, frontal cortex, and amygdala. Besides 5HT, the toxin also depleted DA at the site of injection, an additional damage that could be prevented by pre-treatment with the DA reuptake inhibitor nomifensine. This 5HT lesion led to moderate anxiogenic effects in the EPM. Also, shock-induced ultrasonic vocalization, but not active avoidance in the shuttle box was affected. In the OF, behavior in the drug-free state appeared normal, at least when comparing groups. Lesion effects became visible in the correlative analyses, since center avoidance was correlated with 5HT. Also, behavioral differences between lesion and control animals were disclosed when challenging them with MDMA, a drug which acts largely via serotonergic mechanisms.

The neurochemical results from the first experiment show substantial reductions of 5HT with all three neurotoxic doses (10–50 μg; see also Chia et al., 1999). 5HT was not only decreased at the site of injection, namely the ventral striatum, but also in the neostriatum, frontal cortex, and amygdala. The potency of the lesion was similar in the striatal structures and the frontal cortex, and was less expressed in the amygdala. With respect to dose-response relations, 10 μg were clearly less effective than the two higher doses, whereas these latter doses led to 5HT depletions, which did not differ from each other statistically.

Since 25 μg of 5,7-DHT proved to be neurotoxically effective, we selected this dose for the second experiment and administered it in 1 μl instead of 2 μl, expecting that this might limit extrastriatal 5HT damage (Bures et al., 1983). However, reducing the volume did not avoid depletions of 5HT outside the ventral striatum. Others also obtained rather distal lesion, for example, additional cortical 5HT loss when injecting the toxin into the amygdala (Sommer et al., 2001). In our case, neostriatal 5HT damage might have been due to ascending toxin diffusion along the injection cannula (Bures et al., 1983). Furthermore, it is possible that 5HT depletions in the frontal cortex or even in the amygdala were due to 5,7-DHT damaging 5HT fibers of passage. Also, 5HT fibers, which project directly from the ventral striatum to the amygdala (Heimer et al., 1991) could be destroyed by the toxin. Alternatively, the toxin might have damaged distal sites by retrogradely damaging the raphé nuclei. Unfortunately, this could not be verified, since the raphé were not analyzed in this study. However, the hypothesis about retrograde raphé damage does not explain why 5HT loss was much less severe in the amygdala (Mobini et al., 2000). Finally, one can assume, that the toxin entered the lateral ventricles, since the site of striatal injection was relatively close to them (i.e. around 800 μm). However, it has to be noted, that studies with ventricular 5,7-DHT injections require much higher toxin doses to produce effective lesions, i.e. 300–400 μg (Hall et al., 1999; Lehmann et al., 2000).

Besides 5HT, DA was also significantly reduced in the ventral striatum in case of the two higher toxin doses (exp.1). Others had found that 5,7-DHT injections spared DA neurons, or that pretreatment with desipramine was sufficient to protect noradrenergic as well as DA neurons from the toxin (Baumgarten and Lachenmayer, 1972; Fletcher et al., 1999; Rex et al., 2003). This pattern is not supported here. Possibly, the site of injection is critical since we injected the toxin into a brain region that is rich in DA terminals (Ferre et al., 1994; Phelix and Broderick, 1995). In order to prevent such DA depletions, we additionally administered the DA reuptake inhibitor nomifensine, which was effective to prevent depletions of DA. These results show that 5,7-DHT can also damage DA, at least when injected in doses of 25–50 μg into the ventral striatum, and this effect can be abolished when inhibiting DA transporters by nomifensine. On the other hand, nomifensine treatment diminished 5HT damage in a dose-dependent way, possible since nomifensine might also partially inhibit 5HT transporters (Suarez-Roca and Cubeddu, 2002). To limit this unwanted 5HT effect, we decided to use the lower dose of nomifensine in the final experiment. There, we replicated the prevention of DA damage, now additionally including data on neostriatal DA, which had not been tested in the second experiment because of technical problems.

In the final experiment, behavioral tests were applied to investigate functional consequences of 5HT damage. In the EPM, we obtained only moderate differences between 5HT lesions and controls. It should be noted, however, that these results are based on two-tailed testing, since we refrained from applying directed hypothesis due to inconsistencies in the literature. Also, our 5HT lesions were only partial, that is, they might not have been substantial enough to decrease extracellular 5HT levels (Hall et al., 1999). Furthermore, we obviously had one outlier with respect to EPM behavior (see Fig. 1). When excluding this animal, the residual lesion group showed significantly less open arm time than the control group. This avoidance of the open arms can be interpreted as an anxiogenic effect (Dawson and Tricklebank, 1995; Rodgers et al., 1997). The latency measure supports this conclusion (Borta et al., 2006; Ho et al., 2004), since animals with lesions showed increased latencies to enter the distal parts of the open arms. Furthermore, these rats showed a trend for less arm entries, which is often taken as an index of locomotor activity. Thus, the anxiogenic effects suggested above might be attributed to a general decrease in behavioral activity. This conclusion, however, is not supported by the OF data (see below) where undrugged locomotor activity in rats with lesions did not differ from control levels.

When comparing the first with the second EPM test, the expected decrease in open arm time was observed in the control group. This typical experience-dependent decline has been interpreted as the expression of an acquired phobic-like response to the open arms (File et al., 1993), or the absence of an approach/avoidance conflict in the re-test (Rodgers and Shepherd, 1993). In the lesion group, such a decrease of open arm time was not found on the second test day. This may reflect a floor-effect, since declines of open arm time from EPM 1 to 2 have been found to be less expressed or even absent in animals with low open arm time during EPM1 (Schwarting and

Pawlak, 2004). Also, the number of total arm entries decreased in the control group, which might reflect increased familiarity with the test situation on the second day (Dawson et al., 1994). The lesion group did not show such a reduction of locomotion, which again might be due to a floor effect, although other factors (motivational, emotional, cognitive) cannot be excluded.

Besides EPM behavior, we also measured active avoidance in a shuttle box as a test of fear-motivated behavior. Based on Ho et al. (2002) we expected that rats with ventral striatal 5HT lesions might show deficits in active avoidance performance. Also, Handley (1995) provided evidence for a role of 5HT mechanisms here. In our study, there were no effects of 5HT lesions on active avoidance behavior, which might be due to (A) 5HT is not critical here, (B) the lesions were not substantially enough, or (C) lesions effects in different brain sites cancelled each other out with respect to active avoidance.

During the active avoidance test, USV was also measured since it is known that rats emit so-called 22 kHz vocalization in aversive situations, like confrontation with predators (Blanchard et al., 1991) or exposure to aversive stimuli, like electric shock (Borta et al., 2006; De Vry et al., 1993; Jelen et al., 2003; van der Poel and Miczek, 1991; Wöhr et al., 2005). As expected, our rats started to vocalize during the active avoidance task, and the vast majority of these ultrasonic calls were in the 22 kHz range. Also, the rats vocalized predominantly during the inter-stimulus intervals, but only rarely during tone/shock presentation (see also Wöhr et al., 2005). It has been suggested (Jelen et al., 2003) that calls during such inter-stimulus intervals reflect a general state of anxiety, as compared to a more specific fear response during CS and shock. Most calls were of long duration (>400 ms) and occurred in bout sequences, all of which confirms data obtained in fear-conditioning tasks (Jelen et al., 2003; Wöhr et al., 2005). These calls did not differ between lesion and control groups with respect to rate or time spent calling. In contrast, rats with lesions emitted calls with a lower maximum frequency and less frequency bandwidth at the end of the calls. Borta et al. (2006) also found frequency differences in rats, which differed in anxiety-related behavior, when they analyzed sound frequencies separately at start and end of calls. van der Poel and Miczek (1991) argued that frequency and its modulation might reflect the subject's affective state, whereas call lengths and intervals were considered to depend on breathing capacity. Our present data indicate that such ultrasonic features can signal treatment-dependent changes that are not detectable in overt behavior. It has been shown before that aversive USV is affected by pharmacological manipulations of 5HT (Jolas et al., 1995; Sanchez, 2003). Our results are probably the first to show that 5HT lesions may affect USV.

Finally, activity in the OF was tested, since 5HT can play a role for locomotor activity, especially in case of the ventral striatum (Carter and Pycock, 1979; Chia et al., 1999; Schwarting and Carey, 1985). The present tests, however, did not yield group differences between lesion and control animals, neither in the novel OF, nor after an injection of saline. Nevertheless, lesion effects were indicated by correlative analyses, since center avoidance was correlated with residual 5HT levels in the frontal cortex and striatum, that is, the lower the 5HT levels there, the higher the avoidance of the center. Unlike to these, 5HT in the amygdala of controls was correlated with center time after subsequent saline injection, that is, when animals became more and more habituated to the testing procedure. This finding indicates that 5HT in the amygdala was also involved (e.g. Zangrossi and Graeff, 1994), but that its role differed in an experience-dependent manner from those of the other structures. Since center avoidance has served as a measure of anxiety-related behavior (Hall, 1934; Prut and Belzung, 2003; Treit and Fundyus, 1989), these results seem to support those from the EPM where we also obtained anxiogenic lesion effects. It has to be noted, however, that avoidance of the center is dependent on the size of the OF or the level of illumination (Prut and Belzung, 2003). Since we used a rather small OF (floor space 41 cm × 41 cm) and tested under low levels of illumination (28 lx red light), the center measures may not have been determined solely by anxiety.

Unlike center time, locomotion and rearing were rather inconspicuous in the lesion group, possibly since effects on locomotor activity require more substantial lesions than those, which are sufficient for its anxiogenic effects. Evidence from other lesion models (especially with the DA neurotoxin 6-hydroxydopamine), however, have shown that behavioral deficits can become revealed despite subtotal lesions, given that the residual transmitter system is challenged, for example, by administration of d-amphetamine which acts via release of DA (Hefti et al., 1980; Schwarting and Huston, 1996). The same strategy was applied here. Instead of d-amphetamine, MDMA was used, since this drug acts largely via 5HT mechanisms, namely by stimulating 5HT release and by inhibiting its reuptake (Cole and Sumnall, 2003; Lyles and Cadet, 2003). In normal rats, MDMA can enhance locomotor activity together with reduced rearing (Kehne et al., 1996; Brennan and Schenk, 2006). In our case, MDMA did not reduce rearing activity. This may reflect a floor effect, since rearing activity was already low due to repeated testing. On the other hand, stimulatory effects on locomotor activity were observed in control rats, and these effects were clearly less expressed in the lesion group (see also Kehne et al., 1996). Since intracellular 5HT is less available in rats with lesions, a reduced effectiveness of MDMA is plausible, which is supported by our correlative analyses showing that the drugs' stimulatory effectiveness declined with increasing degrees of 5HT depletion. Besides locomotion, center time and entries of control rats were also increased by MDMA, an effect that was less expressed in lesioned rats. Assuming that these measures reflect anxiety-related behavior (see also above) one could argue that the present dose of MDMA had stimulatory and anxiolytic effects in intact rats (see also Ho et al., 2004; Ando et al., 2006), and these effects were abolished by the 5HT lesion.

Overall, the EPM data and center avoidance in the OF indicate that our 5HT lesion acted in an anxiogenic way. Previous studies provided rather mixed results regarding anxiety, which may be due to factors such as local degree and anatomical extent of lesion: Studies with more severe 5HT lesions (81–99%) led to anxiolytic effects (Briley et al., 1990; Olausson et al., 2001), rather moderate lesions (20–48%; Harro et al., 2001; Gurtman et al., 2002) showed anxiogenic effects, and intermediate lesions showed no effects (54–81%; Andrade and Graeff, 2001; Sommer

et al., 2001; Netto et al., 2002; Rex et al., 2003). Interestingly, effects on anxiety-related behavior became also visible there, when rats were challenged with additional stressors (Andrade and Graeff, 2001; Netto et al., 2002). In these studies, lesion techniques that lead to global losses of central 5HT were used. It has to be noted, however, that 5HT seems to play anatomically distinct, and sometimes, opposite modulatory roles with respect to anxiety (Deakin and Graeff, 1991; File et al., 1996). Although our striatal injections failed to be anatomically selective despite several methodological attempts, they still were most substantial in the more anterior forebrain (frontal cortex, striatum) than posterior to it (amygdala).

Together, our data show that intrastriatal 5,7-DHT can be used to destroy 5HT innervation in the anterior forebrain. Here, great care has to be taken to induce a lesion that is not only substantial, but also neurochemically selective. At least in case of the striatum, is seems extremely difficult to anatomically limit this damage to the site of injection. Such neurochemically specific lesions can have anxiogenic effects, but it cannot yet be concluded whether these effects depend on damage in one critical brain area, here the ventral striatum, or whether they are a consequence of damage in multiple sites.

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Research report

Behavioral and neurochemical consequences of multiple MDMA administrations in the rat: Role of individual differences in anxiety-related behavior

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Abstract

Using the elevated plus-maze (EPM), Wistar rats can be distinguished into high (HA) or low anxiety (LA) subjects. These differences seem to reflect traits, since HA and LA rats vary also in other anxiety-dependent tasks, neurochemical mechanisms, and psychopharmacological reactivity, including lasting consequences after single treatment with 3,4-methylenedioxymethamphetamine (MDMA). Here, we tested whether multiple MDMA treatments also have subject-dependent effects. Based on routine EPM screening, male Wistar rats were divided into HA and LA subgroups, which received five (i.e. multiple) daily injections of MDMA (5 mg/kg) or saline, followed by a test battery, including a challenge test with MDMA, a retest in the EPM, a novel-object test, and a final neurochemical analysis. Acutely, MDMA led to comparable hyperactivity in HA and LA rats. After multiple MDMA, behavioral sensitization was observed, especially in LA rats. Open arm time during the EPM retest (min0–5) correlated with that of the initial one only in those rats, which had received a single injection of MDMA. Rats with multiple MDMA, especially LA-rats, showed more open-arm time and locomotion during the subsequent 5–10 min of the retest. In a novel-object test, rats with multiple MDMA, again especially LA subjects, showed more exploratory bouts towards the novel object. Neurochemically, multiple MDMA led to moderately lower serotonin in the ventral striatum, and higher dopamine levels in the frontal cortex as compared to single MDMA; these effects were also moderated by subject-dependent factors. Our data show that low-dosed multiple MDMA can lead to behavioral sensitization and outlasting consequences, which affect behavior in the EPM and a novel object task. Detecting such sequels partly requires consideration of individual differences.

Keywords: Individual differences; Anxiety; Sensitization; Elevated plus-maze; MDMA; Ecstasy; Novel-object test; Serotonin

1. Introduction

The amphetamine derivative 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy), which is a substrate for the serotonin (5-hydroxytryptamine, 5HT) reuptake transporter [1], stimulates 5HT release and inhibits 5HT reuptake in the brain. Also, MDMA has similar, but less pronounced effects on dopamine (DA; for reviews see [2–4]). MDMA has become a popular recreational drug, since humans consume it for its acute effects, including euphoria, reduction of negative thoughts, and increased sociability and energy. Regular use of MDMA, however, can lead to cognitive and psychiatric problems, like memory deficits, enhanced impulsivity, impaired decision-making, anxiety disorder, and depression [5–10]. However, results from clinical investigations have been inconsistent, since not all studies reported such deficits [11,12]. Part of these inconsistencies may be due to individual differences, that is, in personality or even pre-morbidity, which may not only affect drug consumption, but also its acute and long-term consequences [13,14]. Such clinical evidence is usually gathered retrospectively, which makes conclusions about causalities extremely difficult. Here, animal models might be helpful, given that dispositions or traits can be determined, which can then specifically be challenged with drug treatments (see also [15]).

Some rat strains, for example, which differ in anxiety-related behavior also vary in their reaction to MDMA [16]. However, systematic differences in anxiety-related behavior are not only found between, but also within strains, i.e. between individual

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subjects. Thus, individual male adult out-bred rats show different levels of open-arm avoidance in the elevated plus-maze (EPM [17]), where rats with high- (HA) or low-anxiety (LA) related behavior can be identified. These differences probably reflect the expression of a trait, since they remain rather stable under appropriate retest conditions [18]. HA and LA rats also vary in other tests where anxiety plays a role, since HA show faster object burying, less active avoidance learning [19], and more aversively motivated ultrasonic vocalization [20]. Furthermore, HA and LA rats were found to vary regarding 5HT tissue levels in the ventral striatum [17], and striatal and cortical levels of the cytokine interleukin-2 [21,22].

Since HA and LA Wistar rats differ in striatal 5HT content, and since MDMA acts via central 5HT, one might expect that the effects of MDMA might differ between them. Previously [23], we applied a single treatment (7.5 mg/kg) and did not detect differences in the acute anxiogenic effects of MDMA, but found long-term, subject-dependent drug effects using a test of active avoidance learning, where LA rats were impaired if they had received MDMA several days before.

In the present study, we examined whether HA and LA rats might also respond differently to a regimen of multiple MDMA administrations (a total of 6 × 5 mg/kg) using a protocol partly derived from [24]. We asked how the 1st injection affects psychomotor activity in an activity box, whether repeated injections might lead to sensitization [24–26], and whether changes in cognitive (novel object test; [27]) and emotional mechanisms (EPM; e.g. [27–29]) may be observed several days after drug discontinuation. Furthermore, we tested whether treatments with MDMA might have anorectic effects, i.e. weight loss [30,31]. Finally, a post-mortem neurochemical analysis was performed to test for alterations of 5HT or DA in the brain (e.g. [3,32]).

2. Methods

2.1. General procedure (for overview see Table 1)

The aim of the study was to test whether and how acute and lasting outcomes of multiple MDMA treatments might differ between HA and LA rats. Therefore, a sample of male Wistar rats was screened in an EPM (termed EPM1) to identify HA and LA rats. These were then apportioned to two treatment groups, which received either 5 daily injections of saline followed, 3 days later, by a single injection of MDMA (group termed S-MDMA), or 5 daily injections with MDMA also followed by the challenge test with MDMA (group termed M-MDMA). Since both treatment groups consisted of HA and LA subjects, the design resulted in a total of four sub-groups: S-MDMA HA, S-MDMA LA, M-MDMA HA, and M-MDMA LA. After the drug treatment phase, all rats were again tested in the EPM (termed EPM2), and then in a novel object test. Finally, brain tissue samples were taken and analyzed neurochemically.

2.2. Animals

Adult male Wistar rats (Harlan Winkelmann, Germany) weighing 242–275 g on the 1st day of handling were used. They were housed individually in acrylic cages (26 cm × 18 cm × 42 cm) with food and water available *ad libitum* and a 12/12 h light/dark cycle. On several days before, during and after drug treatment, the rats were weighed, and on 3 consecutive days (5 min each day) prior behavioral testing, they were handled. Also, water consumption was measured during the phase of drug treatment by daily weighing the water bottles provided in the home cages.

2.3. Pre-drug behavioral screening in the elevated plus-maze (EPM1)

Five days after arrival in the lab, the animals were screened for 5 min in the EPM, a pharmacologically validated and well-established animal model of anxiety (for review see [33]). The EPM used consisted of two opposed open arms (50 cm × 10 cm; surrounded by a small rim, 4 mm × 8 mm), two opposed enclosed arms with no roof (50 cm × 10 cm × 40 cm), and an open square (10 cm × 10 cm) in the center. The maze was elevated 50 cm above the floor and was monitored by a video camera from above, which fed into a VCR and a PC running a video-image analyzing software (EthoVision, Noldus, The Netherlands). Testing was conducted under white light (30 lx in the center), and was started by placing the rat into the center. In contrast to our previous observational evaluations the measurement of open-arm time was obtained using an automated system (EthoVision). Open-arm time (as the measure of anxiety-related behavior) and total distance travelled (as a measure of locomotor activity) were recorded. The reliability of the automated analysis was verified by correlating software evaluation with that from our routine observational technique, performed by a skilled observer (Spearman's rho = .966, $p < .001$).

The total amount of open-arm time spent served as the criterion to assign animals to the HA and LA sub-groups (according to [19,23]), that is, based on time spent in the open arms of the maze, the animals were ranked and subjects above and below the median were termed LA and HA rats, respectively. These subjects were then apportioned to the two treatment groups (S-MDMA, M-MDMA), so that both treatment groups consisted of HA and LA rats.

2.4. Drug-treatment

Two days after the initial EPM test, rats received daily subcutaneous injections of either saline (S-MDMA; $n = 19$; 0.9% NaCl), or (±)-MDMA hydrochloride (M-MDMA; $n = 20$; 5 mg/kg, Lipomed, Switzerland, dissolved in 1 ml/kg 0.9% NaCl) on 5 consecutive days. After a two-days break, all rats received the same dose of MDMA.

2.5. Post-drug tests

2.5.1. Activity box

A small open field (41 cm × 41 cm × 40 cm) was used, which was monitored by an automated activity monitoring system (TruScan, Photo beam Sensor-E63-

Table 1
Experimental design

Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
S-MDMA M-MDMA	A	H	H	H		EPM1		S M	S M	S M	S M	S M			M		EPM2		N1	N2				T

Abbreviations: S-MDMA: single administration of MDMA; M-MDMA: multiple administrations of MDMA; A: arrival; H: handling; EPM: elevated plus-maze (EPM1: test duration 5 min; EPM2: test duration 10 min); M: administration of MDMA (5 mg/kg, s.c.) immediately followed by behavioral monitoring in the activity box (60 min); S: administration of saline immediately followed by monitoring in the activity box (60 min); N1: novel object-test, 1st day: habituation to the test apparatus; N2: novel object-test, 2nd day, 1st and 2nd trial (two identical objects, and one familiar and one novel object, respectively; ITI = 18 min); T: tissue sampling. Empty fields indicate days without treatment and tests.

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22, Coulbourn Instruments, USA). Behavior was tested under red light (28 lx in the center) for 60 min immediately after injection of saline or MDMA. The following parameters were evaluated: Rearing (number of times the rat reared on its hind legs), and locomotion (distance traveled in cm). This distance was expressed either as a total score, or separately as distance traveled in the center (27 cm × 27 cm), or the margin area of the activity box.

2.5.2. Elevated plus maze (EPM2)

Two days after the last test in the activity box, the rats were retested in the EPM (EPM2). The procedure was identical to that of the pre-drug test, except that the rats were now tested for 10 instead of 5 min: Behavior in min0–5 of EPM2 was compared with that of the screening test (EPM1). Furthermore, by elongating the EPM2 test by 5 min we wanted to gain more insight into the possible consequences of multiple MDMA treatments, since it is known that behavior shifts with time in this test. Thus, the initial minutes of testing seem to be largely determined by unconditioned emotional factors, whereas later on, behavior seems to reflect experience of the test situation (for review see [34]). In order to minimize possible long-term consequences of the first EPM test, we abstained from also applying the extended test period there.

2.5.3. Novel object test

Two days after EPM2, the rats were tested in the novel object test, which was performed in an open field (58 cm × 58 cm × 39 cm). Behavior was tested under red light (28 lx in the center) and monitored by a video camera from above. Each rat was submitted to one habituation session for 10 min to explore the apparatus without objects. Novel object testing was performed 1 day after this habituation session and consisted of two trials (according to [35]). In the 1st trial, rats were exposed to two identical novel objects. In the 2nd trial, they were exposed to one object from the previous trial (familiar object) and a new one (novel object). As our objects, we used a red iron block (5 cm × 5 cm × 8 cm) and a solid glass pillar (diameter: 6 cm, high: 8 cm). The kind of object presented during the 1st as well as its position during the 2nd trial were counterbalanced and randomly permuted in LA and HA rats. Trial duration was 3 min with an inter-trial interval of 18 min. Object exploration was measured when the rat directed its nose towards the object within a distance of 2 cm from it. This behavior was scored in terms of the number of events (frequency of exploration) and time spent (total exploration time).

2.6. Neurochemical analysis

The animals were lightly anaesthetized with Narkoren (Merial, Germany) and decapitated. The brains were quickly removed and the ventral striatum and frontal cortex were dissected out bilaterally, homogenized in 0.05 M perchloric acid, and stored at −80 °C. The samples were analyzed for their contents of 5HT, 5-hydroxyindole acetic acid (5HIAA), DA, and dihydroxyphenylacetic acid (DOPAC) using high-performance liquid chromatography with electrochemical detection (HPLC-EC; Antec Leyden BV, Netherlands). These biogenic amines were separated on a Nucleosil 100-5 C_{18} column (125 mm × 4 mm, particle size 5 μm, Macherey-Nagel, Germany) using a mobile phase containing 35 ml/l acetonitrile, 140 mg/l octanesulphonic acid, 100 mg/l Na_2EDTA, and 6 ml/l triethylamine (pH 2.95). The detector potential was set at 600 mV relative to an Ag/AgCl reference electrode.

2.7. Statistical analysis

Data are presented as means ± S.E.M. For statistical analysis, all data were initially examined using the Shapiro-Wilk procedure [36] for possible deviations from normality of distribution, and Mauchly's test for the assumption of sphericity. Since these requirements were not fulfilled in several cases, including behavioral and neurochemical measures, we decided to apply non-parametric statistics throughout (Wilcoxon Rank-Sum test, Wilcoxon Signed-Rank test, test of Lam & Longnecker, Spearman's correlation test; [37–39]). Furthermore, a non-parametric marginal model procedure allowing statistical analysis of non-parametric longitudinal data samples with discrete and tied values (for a survey article, see: [40]; for a comprehensive introduction to theory and application, see: [41]) was applied to examine data samples from the activity box, body weight, and water intake curves. All quantitative analyses were performed with the software packages R 2.4.1. and SPSS 12.0.

Regarding the interpretation of statistical results, we abstained from using the procedure of null hypothesis significance testing (NHST) due to the following reasons: The NHST, often implying values of .05 as the golden standard for dividing findings into scientifically supported and not supported statements, has been fiercely criticized for its theoretical inconsistency and incompatibility with concepts as size of effect or test power [42,43]. Therefore, we chose not to apply NHST and the dichotomous division in the categories "significant" and "not significant". Instead, Fisher's *p*-value, as a continuous measure for the strength of evidence against the Null hypothesis [42], incompatible with the aforementioned (or any other) categories, was applied. Thus, for all tests exact *p*-values are reported, and the results are neither categorized as "significant/non-significant", nor as "trends". *P*-values of two-tailed tests (exact *p*-values in case of Wilcoxon's tests; [39]) were used in all cases except 5HT levels, where one-tailed tests were used, since we expected depletions with our MDMA regimen. In figures and tables, we did not use conventional symbols (such as *, or ***), since they imply that significance tests have been used. It should be noted that even though Fisher's *p* allows subjective interpretation of the obtained data, the space for interpretation is strongly limited by the exact *p*-value itself and the whole body of evidence presented [42].

Fisher's *p*-values could be extended by reports on the empirical size of effect [44]. However, as reported in [45] reported, small deviations in data distribution could strongly bias the conventional measures of effect size. Since several of the present data sets were not normally distributed, we did not calculate effect sizes, as it is not reliably applicable to non-parametric data sets.

3. Results

3.1. Screening in the elevated plus-maze and group assignments

In the initial screening test, the later treatment groups (i.e. S-MDMA and M-MDMA) did not differ with respect to open-arm time (S-MDMA: 31.2 ± 7.4; M-MDMA: 34.8 ± 10.0; mean ± S.E.M.; $p = .928$). Therefore, it can be assumed that our procedure of assignment led to treatment groups, which were similar with respect to basal anxiety-related behavior. These groups also did not differ with respect to locomotor activity in the EPM (S-MDMA: 12.72 ± .85; M-MDMA: 12.48 ± .64; $p = .496$).

The two treatment groups consisted of sub-groups with high or low anxiety-related behavior. Open-arm times of these sub-groups were: M-MDMA HA: 5.7 ± 2.7, M-MDMA LA: 63.8 ± 15.0; S-MDMA HA: 7.5 ± 2.8, S-MDMA LA: 57.6 ± 9.3. In both treatment groups, HA rats showed substantially less open-arm time than LA rats (p-values < .001). In the S-MDMA group, locomotor activity did not differ between HA (11.8 ± 1.45) and LA rats (13.8 ± .76; $p = .447$), whereas in the M-MDMA-group, it was higher in LA (13.9 ± .52) than HA rats (11.1 ± 1.0; $p = .009$).

3.2. Effects of acute and repeated MDMA administration in the activity box

3.2.1. 1st treatment

Compared to saline injection (group S-MDMA), MDMA treatment led to enhanced total locomotion (Fig. 1; $p < .001$) both, in the center (Fig. 2; $p < .001$) and the margin area

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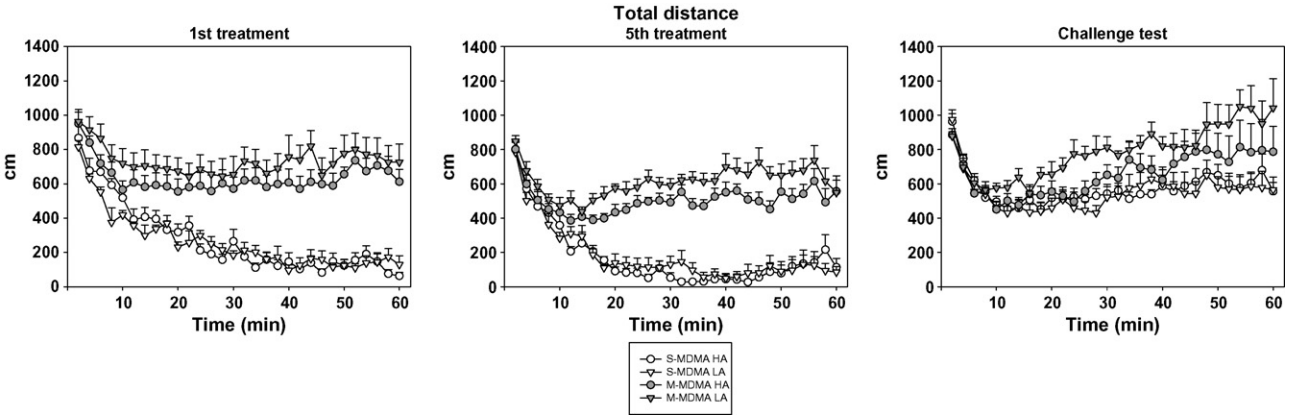

Fig. 1. Locomotor activity (total distance traveled in cm/2 min; mean + S.E.M.) of rats belonging either to the HA- (circles) or LA-subgroup (triangles). Rats with multiple MDMA treatments (M-MDMA) received five daily injections of MDMA (5 mg/kg, s.c.; gray symbols) and behavior was tested after the first (left) or fifth (middle) injection. S-MDMA rats (open symbols) received saline injections during this period. Three days after the fifth injection, all rats underwent a challenge test with 5 mg/kg MDMA (right figure).

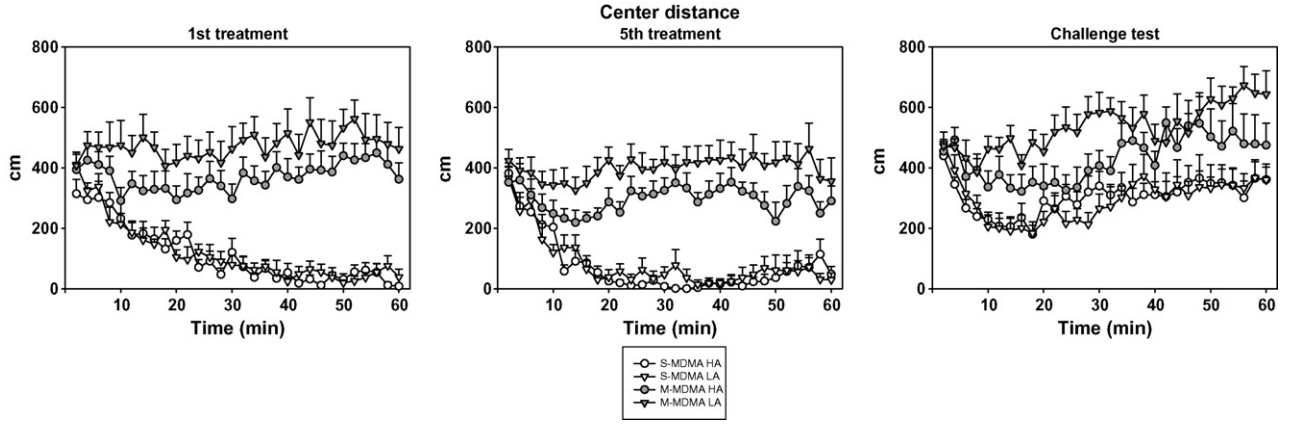

Fig. 2. Locomotion in the center of the activity box (distance traveled in cm/2 min; mean + S.E.M.) of rats belonging either to the HA- (circles) or LA-subgroup (triangles). Rats with multiple MDMA treatments (M-MDMA) received five daily injections of MDMA (5 mg/kg, s.c.; gray symbols) and behavior was tested after the first (left) or fifth (middle) injection. S-MDMA rats (open symbols) received saline injections during this period. Three days after the fifth injection, all rats underwent a challenge test with 5 mg/kg MDMA (right figure).

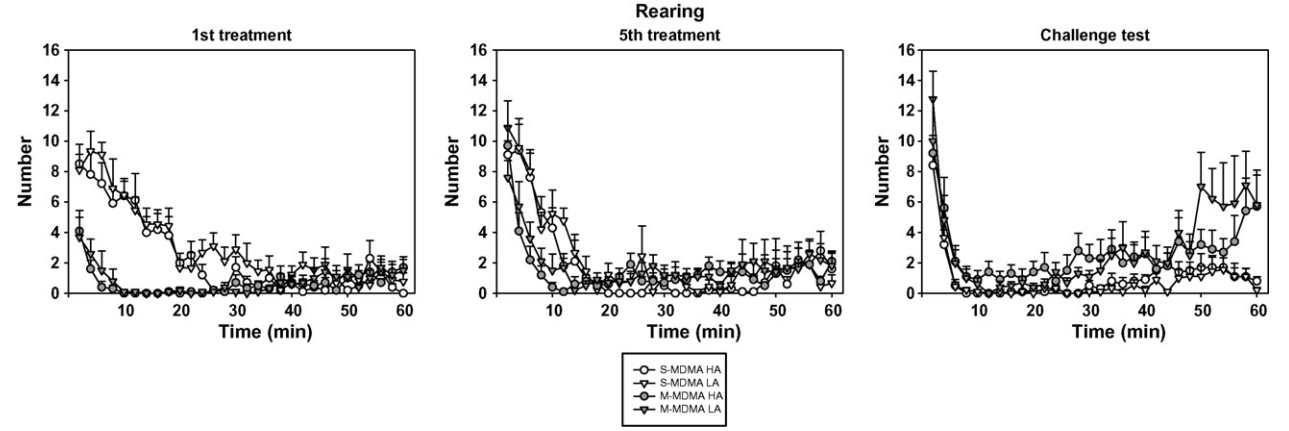

Fig. 3. Rearing behavior in the activity box (number/2 min; mean + S.E.M.) of rats belonging either to the HA- (circles) or LA-subgroup (triangles). Rats with multiple MDMA treatments (M-MDMA) received five daily injections of MDMA (5 mg/kg, s.c.; gray symbols) and behavior was tested after the first (left) or fifth (middle) injection. S-MDMA rats (open symbols) received saline injections during this period. Three days after the fifth injection, all rats underwent a challenge test with 5 mg/kg MDMA (right figure).

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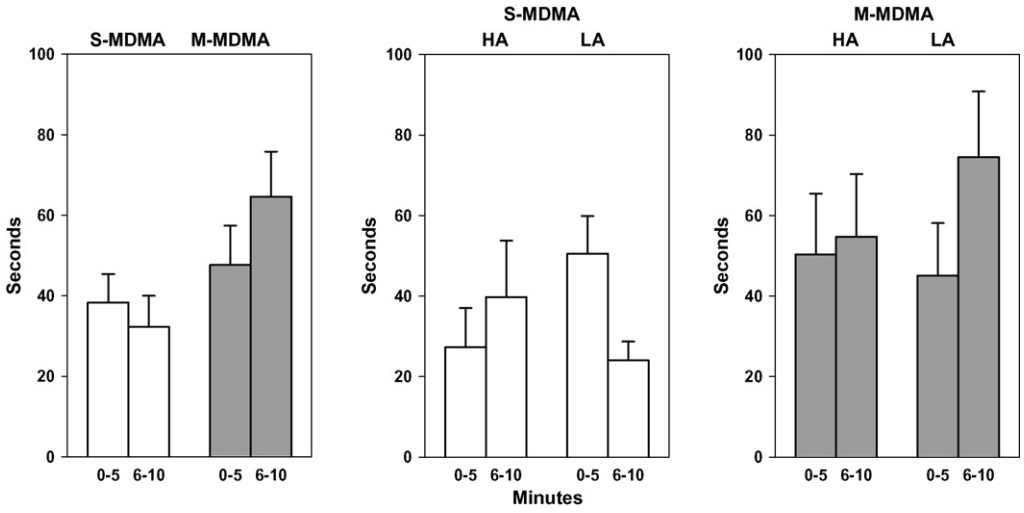

Fig. 4. Open-arm time (in seconds; mean ± S.E.M.) in the elevated plus-maze during two consecutive time periods of 5 min. In the left part, data from rats with multiple (M-MDMA, grey bars) or single MDMA treatments (S-MDMA, open bars) are presented irrespective of HA- and LA-subgroups. The middle and right part shows the same data subdivided into those from the HA- and LA-subgroups.

($p = .008$, results not shown). These effects became prominent after about 5–10 min of observation (interactions between treatment and time; $p < .001$). Rearing activity was decreased by MDMA (Fig. 3; $p < .001$), and this effect was observed during the initial 10–20 min of observation (interaction between treatment and time; $p < .001$), that is, when saline-injected rats were most active. No differences between HA and LA rats, and no interactions between treatment and sub-groups were found (all p-values > .100).

3.2.2. 5th treatment

Similar to the 1st test, locomotion was increased by MDMA (all p-values < .001), an effect which became prominent after about 10 min. Regarding rearing, there was no main effect of treatment ($p = .173$), but an interaction between treatment and time ($p < .001$), since multiple MDMA decreased rearing during the initial 10 min. Overall, LA rats showed more total locomotion ($p = .015$) and more locomotion in the center ($p = .017$). In case of total locomotion, this effect was largely due to LA rats with multiple MDMA treatments (interaction between treatment and sub-groups: $p = .054$). In case of rearing, no differences between HA and LA rats were found.

3.2.3. Challenge test

Two days after the 5th treatment, all rats were challenged with MDMA. Again, rats which had undergone multiple MDMA treatments before showed enhanced total ($p = .002$) and center locomotion ($p < .001$), whereas their locomotion in the margin area was decreased ($p = .047$). Furthermore, there were moderate interactions between treatment and sub-groups in case of center locomotion ($p = .063$) and total locomotion ($p = .058$), since LA rats with multiple MDMA tended to show more locomotor activity than HA rats. Rearing was increased in rats with multiple MDMA ($p < .001$), an effect, which appeared to be more pronounced during the 2nd half of testing. Rearing did not differ between HA and LA rats.

3.3. Effects of multiple MDMA treatments on plus-maze behavior (EPM2)

When comparing the two treatment groups, no difference in open-arm time ($p = .682$; Fig. 4) or locomotor activity ($p = .336$; Fig. 5) during min0–5 of the second plus-maze test was found. Treatment effects became observable, however, when correlating open-arm time (min0–5) of this post-treatment test to that of the initial screening test (EPM1): In the S-MDMA group, there was a positive correlation between the two tests (rho = .522, $p = .022$; data not shown), indicating substantial retest reliability of our measure of anxiety-related behavior. Analysis of the sub-groups showed that this effect was largely due to the LA group (rho = .700, $p = .036$; HA rats: rho = .399, $p = .254$). In rats, which had undergone multiple MDMA injections (M-MDMA), there were no substantial correlations, neither in the total group (rho = .061, $p = .799$), nor in the sub-groups (HA: rho = −.198, $p = .583$; LA: rho = .345, $p = .328$). Finally, there were no correlations of locomotor behavior between EPM2 and EPM1 (S-MDMA: rho = .056, $p = .819$, M-MDMA: rho = .120, $p = .613$).

Analysis of behavior during min6–10 of the post-treatment test yielded further effects of multiple MDMA treatments, since M-MDMA rats now showed more open-arm time (Fig. 4; $p = .022$) and more locomotor activity (Fig. 5; $p = .028$) than S-MDMA rats. When examining HA and LA rats within these treatment groups, we found that the effect of multiple MDMA administrations was largely due to LA subjects: In these rats (M-MDMA LA), open-arm time increased during min6–10 (as compared to min05; $p = .049$), whereas it decreased if such rats had received only a single injection of MDMA (S-MDMA LA, $p = .039$). No such effects occurred in HA rats (S-MDMA HA: $p = .922$; M-MDMA HA: $p = .945$). Also, the M-MDMA LA sub-group showed more open-arm time during min6–10 than the S-MDMA LA counterpart ($p = .008$), whereas no such effect was obtained between respective HA rats ($p = .490$). Locomotor activity during min6–10 (as compared to min0–5) decreased

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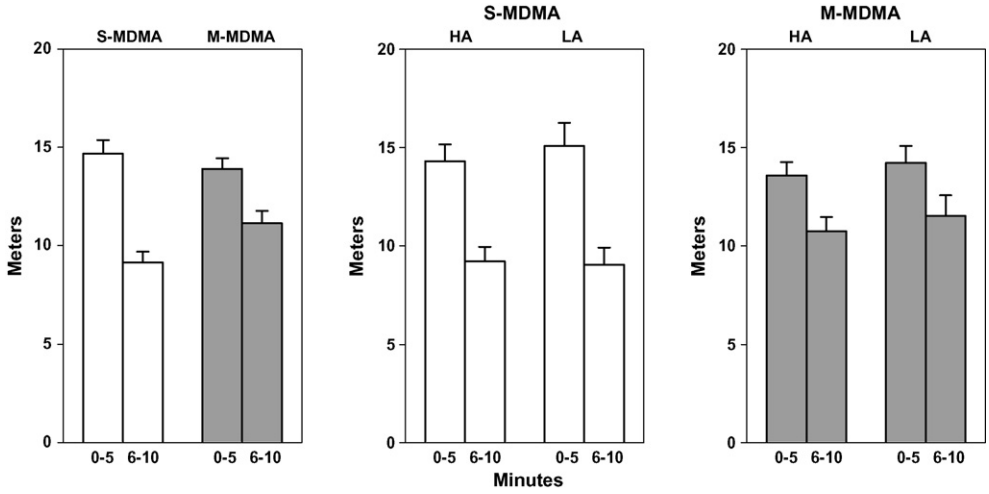

Fig. 5. Distance moved (meters; mean ± S.E.M.) in the elevated plus-maze during two consecutive time periods of 5 min. In the left part, data from rats with (M-MDMA, grey bars) or single MDMA treatments (S-MDMA, open bars) are presented irrespective of HA- and LA-subgroups. The middle and right part shows the same data subdivided into those from the HA- and LA-subgroups.

within sub-groups (p-values between .002 and .006), with the least effect in the M-MDMA LA group ($p = .064$).

3.4. Novel object test

3.4.1. 1st trial

Here, two identical novel objects were presented in the open field. Exploration times (Table 2a) did not differ between treatment groups (S-MDMA vs. M-MDMA: $p = .771$). Also, there were no differences when comparing treatments within HA rats ($p = .912$), or within LA rats ($p = .447$), respectively. The frequencies of explorations (Table 2b) tended to be higher in M-MDMA rats ($p = .074$), and when comparing these frequencies between sub-groups, the same tendency was found between LA rats (LA-M-MDMA vs. LA-S-MDMA: $p = .075$) but not HA rats ($p = .491$).

3.4.2. 2nd trial—exploration time

Here, one of the previous objects and a novel one were presented. Compared to the 1st trial, the total time of exploration (i.e. of both objects) did not change in any group (all p-values > .100). When comparing the novel vs. the familiar object, both treatment groups showed more exploration of the novel than the familiar object (M-MDMA: $p < .001$; S-MDMA: $p = .005$). This effect was also observed in the sub-groups (p-values between .010 and .001), except for S-MDMA HA rats ($p = .301$).

3.4.3. 2nd trial—exploratory frequency

Compared to the 1st trial, the frequencies of exploration did not change in the 2nd trial in S-MDMA rats ($p = .270$), whereas they declined in the M-MDMA group ($p < .001$) and this effect was observed in both, HA ($p = .016$) and LA rats ($p = .012$). Within the 2nd trial, the exploration frequencies of the novel vs. the familiar object did not differ in the S-MDMA group ($p = .751$). In contrast, M-MDMA rats showed higher exploratory frequency of the novel object ($p = .022$), which was largely due to M-MDMA LA rats ($p = .010$), since there was no effect in M-MDMA HA rats ($p = .719$). Exploratory frequencies of the familiar object did not differ between M-MDMA and S-MDMA, or between the sub-groups (all p-values > .100).

3.5. Body weight and water intake

Before multiple MDMA treatments, body weights (Fig. 6) increased ($p < .001$) but did not differ between HA- and LA-

Table 2a
Novel-object test

Treatment sub-group	Trial 1:	Trial 2:		
	Exploration time (two objects)	Exploration time (both objects)	Exploration time (familiar object)	Exploration time (new object)
S-MDMA	49.01 ± 2.90	50.22 ± 5.86	17.17 ± 2.28	33.06 ± 4.76
HA	51.27 ± 4.76	46.64 ± 9.25	18.05 ± 3.61	28.59 ± 7.32
LA	46.50 ± 3.16	54.20 ± 7.25	16.19 ± 2.86	38.02 ± 5.90
M-MDMA	51.74 ± 2.07	51.35 ± 4.54	14.69 ± 1.85	36.66 ± 3.62
HA	52.20 ± 3.27	49.66 ± 6.48	16.94 ± 3.03	32.73 ± 4.20
LA	51.28 ± 2.69	53.03 ± 6.66	12.45 ± 2.03	40.59 ± 5.84

S-MDMA: single administration of MDMA; M-MDMA: multiple administrations of MDMA. Exploration time is given in seconds. Values reflect means ± S.E.M.

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Table 2b
Novel-object test

Treatment sub-group	Trial 1:	Trial 2:		
	Exploratory frequency (two objects)	Exploratory frequency (both objects)	Exploratory frequency (familiar object)	Exploratory frequency (new object)
S-MDMA	19.74 ± 1.22	18.00 ± 1.40	8.79 ± .96	9.21 ± .89
HA	20.40 ± 1.83	18.10 ± 2.19	9.40 ± 1.25	8.70 ± 1.14
LA	19.00 ± 1.65	17.89 ± 1.84	8.11 ± 1.53	9.78 ± 1.46
M-MDMA	22.30 ± .83	17.80 ± 1.26	7.80 ± .71	10.00 ± .79
HA	21.70 ± 1.20	18.60 ± 1.45	9.00 ± .89	9.60 ± .92
LA	22.90 ± 1.17	17.00 ± 1.45	6.60 ± 1.01	10.40 ± 1.32

S-MDMA: single administration of MDMA; M-MDMA: multiple administrations of MDMA. Exploration frequency depicts the number of investigatory bouts towards a given object. Values reflect means ± S.E.M.

rats ($p = .858$), or between the later M-MDMA and S-MDMA groups ($p = .431$). On the days of MDMA administration and the day thereafter, there was no general effect of multiple MDMA-treatments (main effect treatment: $p = .731$), but an effect over time (interaction between treatment and time: $p = .011$), since rats in the M-MDMA group gained less weight than in the S-MDMA group towards the end of this observational period. Furthermore, there was an interaction between treatment, time, and sub-groups ($p = .014$), since the effect of multiple MDMA was largely due to the HA group. In the period after drug treatment, body weights of HA rats in the M-MDMA group still lay below those of the other groups, but there were no longer substantial effects of multiple MDMA-treatments ($p = .293$), nor any interactions between treatment, time and sub-groups (all p-values > .100).

Water intake in the home cage was measured during the 24-h periods following drug treatment on days 8–12 (Fig. 7), and it was found that rats in the M-MDMA group consumed more water than those in the S-MDMA group ($p < .001$), but no differences between HA and LA rats ($p = .283$), nor interactions between treatment and sub-groups ($p = .846$) were found.

3.6. Neurochemical analysis

3.6.1. Ventral striatum

One rat from the S-MDMA group (LA sub-group) was excluded from 5HT analysis in the ventral striatum, since its data were extremely low (more than 2.5 standard deviations from the mean) and thus this value was categorized as an outlier. When comparing ventral striatal results in the remaining subjects, we found that 5HT was decreased (Table 3; $p = .041$) in the M-MDMA group, as compared to S-MDMA. The other striatal neurochemical measures did not yield differences between treatments, and there were no differences between LA and HA sub-groups (all p-values > .100).

3.6.2. Frontal cortex

In contrast to the ventral striatum, multiple MDMA treatments did not affect 5HT levels in the frontal cortex (Table 3), as compared to the single treatment (M-MDMA vs. S-MDMA: $p = .161$), whereas DA levels tended to be higher ($p = .095$), and DOPAC/DA ratios tended to be lower ($p = .095$) in the M-MDMA group. When comparing sub-groups, we found that HA rats in the M-MDMA group had lower DOPAC/DA ratios

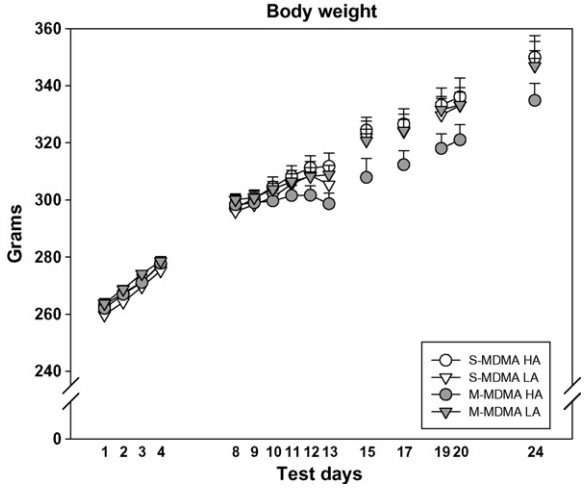

Fig. 6. Body weight (in grams; mean ± S.E.M.) of rats belonging either to the HA- (circles) or LA-subgroups (triangles). M-MDMA rats (grey symbols) received MDMA injections (5 mg/kg, s.c.) on days 8–12 and 15, whereas S-MDMA rats (open symbols) received saline on days 8–12 and MDMA on day 15. For further design details see Table 1.

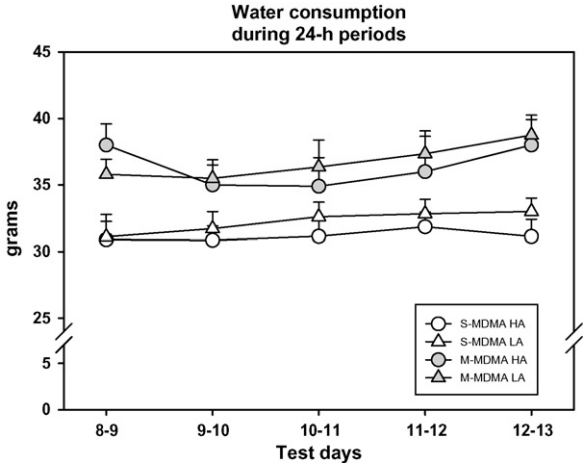

Fig. 7. Water consumption (in grams; mean ± S.E.M.) of rats belonging either to the HA- (circles) or LA-subgroups (triangles). Water consumption was measured during the 24-h periods following treatments administered on days 8–12. M-MDMA rats (grey symbols) received daily injections of MDMA (5 mg/kg, s.c.) during this period, whereas S-MDMA rats (open symbols) received saline.

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Table 3
MDMA effects on neurochemistry

Region	Treatment sub-group	5HT	5HIAA	5HIAA/5HT	DA	DOPAC	DOPAC/DA
Ventral striatum	S-MDMA	1.254 ± .028	.419 ± .014	.337 ± .011	7.958 ± .235	1.145 ± .036	.144 ± .004
	HA	1.271 ± .029	.421 ± .011	.334 ± .012	8.076 ± .213	1.131 ± .035	.141 ± .006
	LA	1.233 ± .052	.415 ± .029	.340 ± .018	7.826 ± .449	1.160 ± .067	.149 ± .004
	M-MDMA	1.189 ± .027	.419 ± .017	.353 ± .009	7.955 ± .211	1.138 ± .048	.142 ± .003
	HA	1.210 ± .037	.436 ± .027	.361 ± .015	7.929 ± .192	1.158 ± .053	.145 ± .003
	LA	1.158 ± .039	.401 ± .021	.345 ± .011	7.980 ± .389	1.117 ± .083	.138 ± .005
Frontal cortex	S-MDMA	.569 ± .020	.135 ± .006	.244 ± .013	.104 ± .018	.077 ± .005	1.062 ± .100
	HA	.567 ± .024	.124 ± .007	.224 ± .018	.082 ± .013	.086 ± .007	1.237 ± .151
	LA	.571 ± .034	.148 ± .010	.265 ± .016	.128 ± .034	.069 ± .006	.868 ± .098
	M-MDMA	.542 ± .017	.143 ± .008	.269 ± .015	.126 ± .017	.077 ± .003	.835 ± .074
	HA	.555 ± .029	.141 ± .008	.260 ± .020	.096 ± .007	.073 ± .004	.861 ± .104
	LA	.529 ± .019	.145 ± .014	.277 ± .023	.155 ± .032	.081 ± .004	.809 ± .110

S-MDMA: single administration of MDMA; M-MDMA: multiple administrations of MDMA. Substance values reflect brain tissue levels in μg/g (means ± S.E.M.).

($p = .023$) than HA rats in the S-MDMA group, together with tendencies for lower levels of DA ($p = .075$) and 5HIAA ($p = .052$). In the S-MDMA group, HA rats tended to have lower 5HIAA levels ($p = .095$) and 5HIAA/5HT ratios ($p = .079$), but higher DOPAC levels ($p = .065$) and DOPAC/DA ratios ($p = .095$) than LA rats.

4. Discussion

The aim of this study was to test whether a low-dosed regimen of multiple MDMA injections might have acute and/or lasting consequences on behavior and physiology in rats, and whether these outcomes might depend on individual dispositions in anxiety-related behavior, which was gauged by a routine screening test in the EPM. Except for the acute psychomotor effects of MDMA, we obtained evidence, albeit mostly moderate, for subject-dependent outcomes in all measures applied, that is, sensitisation to multiple MDMA, weight loss during the phase of drug treatment, subsequent behavioral effects, and forebrain neurochemistry as compared to animals, which had received only a single injection of MDMA. Therefore, the present results point at a critical role of subject-inherent factors.

4.1. Acute behavioral effects of MDMA

The 1st injection of MDMA led to locomotor activation. Furthermore, the drug almost entirely abolished rearing activity during the testing period of 60 min. These results indicate that the present dose led to both, hyperactivity (enhanced locomotion), and hypo-exploration (less rearing), supporting several previous findings ([24,46–50], but see [51,52]). Also, we found enhanced center activity, which is often taken as an index of anxiolytic drug action. Since MDMA can have dose-dependent anxiogenic effects [23,46,47], our data appear to be contradictory, but it should be noted that we used a rather small environment (41 cm × 41 cm) and tested under low levels of illumination, i.e. conditions, under which anxiety may not play a substantial role. Importantly, none of our measures yielded acute differences between HA and LA rats, which supports our previous results [19,23].

4.2. Behavioral activity after multiple MDMA

When tested after the 5th injection, roughly similar locomotor patterns were observed as after the 1st one, that is, more locomotor activity under MDMA than saline. This effect was mainly due to the LA rats.

On the final day of drug testing, when all rats were challenged with MDMA, indices of sensitization were found since locomotor and rearing activity was higher in MDMA pre-treated rats. These effects occurred especially during the second half of the testing period, corroborating the results of [24], who also used a dose of 5 mg/kg, paired its effects with the testing environment (see also [53]), and tested for sensitisation at a comparable time point. Such sensitisation was also observed in several [25,51,53–56], but not all previous studies [57–59]. Differences between studies were attributed to factors like dose, temporal details of the testing regimen, and whether drug experiences where made in the testing environment or a distinct one. Our results add preliminary evidence that individual dispositions may also be a factor, since sensitization effects tended to be more pronounced in LA rats.

4.3. Elevated plus-maze

As our critical test of anxiety we used the EPM, where open arm avoidance is usually taken as the major index of anxiety-related behavior. During the post-treatment test (EPM2), behavior was recorded during a period of 10 min, which is twice as long as in most other experiments (for review see [34]). During the 1st 5 min, that is, the usual testing period, there were no differences in open-arm times or distance traveled between the M-MDMA and S-MDMA groups. Treatment effects became observable, however, when correlating behavior of the post-treatment test to that during the initial screening test (EPM1), since a positive correlation between the two tests was obtained in case of rats, which had received MDMA only

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once. The fact, that this correlation was observed only in the LA but not HA rats, is probably due to low open arm time, i.e. a floor effect, and thus low variability in the latter. In general, the test–retest correlation supports our previous conclusion [18] that EPM behavior, when tested under appropriate conditions, can gauge a trait of anxiety-related behavior. In the S-MDMA group, the expression of this trait was apparently not blunted by the various experiences between the two tests, including several saline and one MDMA injection, and repeated exposures to the activity box. In contrast, the lack of a test–retest correlation in the M-MDMA-group may indicate that multiple MDMA had affected the trait itself, or factors related to its expression.

Further consequences of multiple MDMA-treatments were observed during min6–10, a time-period, which is subsequent to the usual one (5 min). Then, M-MDMA rats spent more time in the open arms and showed more locomotor activity, and especially in case of open-arm time, the effect was due to the LA sub-group. Increased open-arm time in the EPM test, at least during min0–5, is often taken as an index of anxiety-related behavior; based on this assumption, one would again conclude that our multiple MDMA-treatments had anxiolytic consequences. This result is in contrast to a bulk of evidence showing anxiogenic effects with tests like the EPM, social interaction, or emergence tests. Also, effects were obtained with various types of multiple MDMA-treatments, and irrespective of whether they led to 5HT depletions or not ([27,29,48,60–63]; see also [64]). Only a minority of studies found no effects in the EPM [65,66], and one study [67] yielded anxiolytic-like effects, but there MDMA was given during adolescence, a period when MDMA may have partly different consequences than during adulthood, as tested here.

Thus, our data seem to somehow contrast the available rat literature, and also the human literature, since occasional to moderate MDMA use is generally not associated with elevated anxiety, whereas heavier use can have anxiogenic consequences [12,68–70]. However, open-arm time in the EPM is not only determined by anxiety (i.e., avoidance), but also by its conflict with curiosity (i.e., approach), and by cognitive and experiential factors. In the initial minutes, most rats explore both types of arms. Thereafter (around min 3), they spend more time in the closed arms, showing stretch-attend postures towards the open arms, which probably reveal an approach/avoidance conflict [34]. Thus, one could assume that this approach/avoidance conflict was affected by multiple MDMA pre-treatments, leading to an enhanced approach tendency and/or a reduced avoidance tendency. Furthermore, one could assume that M-MDMA rats, especially LA subjects, were somehow more active and did not habituate to the ongoing EPM experience, which prevented the later shift to the closed arms. It should be noted that locomotor activity during the initial screening test had been slightly higher in the M-MDMA LA group, an accidental difference, which may have partly biased the outcome in the second plus-maze test. Locomotor activity and open-arm time, however, are usually considered as independent factors of plus-maze behavior. Therefore, we consider it unlikely that the changes of open arm time in the M-MDMA LA group were due to an initial bias of locomotor activity.

The present data do not allow to draw any firm conclusions on the possible mechanisms, but they show that effects of MDMA pre-treatment can become apparent when prolonging the test period, and when considering individual aspects. Thus, factors related to anxiety-like behavior, which can differ between like individuals (as shown here), or strains (discussed in [16]), seem to modulate the outcome of MDMA-treatments in tests of anxiety. These findings raise the hypothesis that such subject-dependent factors may also be relevant for the variability of psychiatric consequences in human MDMA users.

4.4. Novel object test

We used a well-established object-recognition test, which is generally used to study short-term memory, since preference for the novel object during the 2nd trial requires recognition of the other, now familiar, object. As our critical behavioral indices, durations and frequencies of object exploration were measured. When confronted with two unfamiliar objects during trial 1, there was no overall difference in exploratory time between the M-MDMA and S-MDMA groups, and only a tendency for more exploratory events in rats with multiple MDMA, especially in LA rats. During the 2nd trial, when novel and familiar objects were presented, the duration of exploration showed that the test worked in the expected way, since most sub-groups displayed more exploration of the novel object.

In contrast to exploratory time, the frequency of exploration did not gauge memory of the familiar or preference for the novel object in rats of the S-MDMA group, since they exhibited similar frequencies to both. Rats with multiple MDMA treatments, on the other hand, showed more exploratory events during the 2nd trial. This behavior was largely directed towards the novel object and occurred mainly in LA rats. Since similar tendencies were already observed during the 1st trial, one cannot necessarily conclude that multiple MDMA-treatments specifically affected mechanisms of memory. Alternatively, MDMA may have led to enhanced responsiveness to novelty, or to enhanced impulsivity, which was also discussed as a possible explanation for human deficits in cognitive tasks [10].

In general, our findings agree with previous reports since they show that the novel object task is suitable to gauge effects of MDMA-treatment ([27,60,67], but see [62,71]). Our data add to these findings in showing that changes can occur even with MDMA regimens, which lead to only moderate 5HT depletions. It remains to be tested whether the deficits observed here are lasting: Since we performed the recognition test 3 days after the last MDMA injection, we cannot exclude that the present deficits are only transient.

4.5. Body weight and water intake

Multiple MDMA regimen affected body weight, since weight gain during the last days of, and the day after repeated MDMA-treatment was decreased. This effect was largely due to the HA group. Also, the effect was transient, since it was no longer observed during the days subsequent to multiple MDMA-treatments. In general, these results corroborate previ-

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ous findings in rats [30,67,72,73] and parallel results in humans [74–76]. In rats, these outcomes may be due to several factors, including increased energy consumption, salivation, urination and defecation, and reduced food intake [30,31,77]. Furthermore, weight loss was found to be associated with the loss of 5HT transporters [73], which is interesting since 5HT plays a critical role in the regulation of feeding and metabolism [78,79].

Our data extend these findings in showing that multiple MDMA regimens with lower total doses as those used before [30,67,72,73] at least transiently also lead to weight loss, and that certain individuals, namely HA rats, are especially vulnerable to this effect. Theoretically, these deficits may have been due to an anorectic effect in food intake, acute water losses, or deficits in compensating for the loss of body water. At least the last factor can be ruled out by the presented data, since HA and LA rats showed similar compensatory increases in water intake during the daily periods following drug treatments.

4.6. Neurochemistry

Many previous studies yielded substantial serotonergic depletions in several brain areas, including cortex and striatum ([29]; McGregor et al., 2003). There, more drastic MDMA regimens as used here were applied, including higher and/or more total doses, or multiple injections per day [66,80], and it is known that the neurochemical outcome of MDMA treatments depends on several factors, including dose and frequency of administration [72,81], rat strain [81–83], and housing conditions [84]. In the present study, multiple MDMA treatments with a comparably low drug dose led to a moderate decrease of ventral striatal 5HT and no difference in cortical 5HT (see also [59]) 9 days after the last drug treatment. These findings have to be assessed with caution: Since our experimental design required a comparison group with a single MDMA injection, which might also have led to depletion of 5HT, we cannot rule out that drug effects would have been more pronounced in the multiple MDMA group, if it had been compared to a control group without any drug treatment.

Also, we did not find effects in DA or DOPAC levels in the ventral striatum, which conforms to most studies analyzing long-term effects of MDMA in rats ([85–87]; but see McGregor et al., 2003; [88]). In the frontal cortex, moderately higher DA levels were observed in the M-MDMA group (again, in comparison to single MDMA). Interestingly [24], who used a similar drug treatment regimen as ours, found increased tissue levels of DA in the nucleus accumbens together with a smaller effect in the frontal cortex. Possibly, such MDMA regimens (5 mg/kg) result in metabolic changes in the dopaminergic system, like decreased basal release, decreased degradation, or increased synthesis. Such mechanisms can result in an accumulation of intracellular DA levels, which constitute the major fraction of DA measured in tissue samples as used here.

Between HA and LA rats, we did not find neurochemical differences in the ventral striatum, neither in the M-MDMA, nor the S-MDMA group. Previously, we found lower striatal tissue levels of 5HT in HA than LA rats [17]. These results cannot be compared to the present ones, since the two studies differ with respect to several methodological aspects, including laboratories (Düsseldorf vs. Marburg), details of tissue sampling, and the types of experiences, which the animals had made before being sacrificed. Out of these, the single MDMA challenge might have been critical, since lasting neurochemical changes in striatal serotonergic systems have been observed before, at least with a higher dose as used here [89]. Such consequences may have blunted the serotonergic differences between HA and LA rats. Still, moderate neurochemical differences between these sub-groups were also found in the present study, but they were detected in the frontal cortex, namely between HA rats of the M- vs. S-MDMA group (dopaminergic), and between HA and LA rats of the S-MDMA group (dopaminergic and serotonergic). The mechanisms of these effects cannot be explained by the present findings, since they may reflect basal differences between HA and LA rats as such, and/or sub-group-dependent reactions to MDMA and its consequences. Irrespective of these unresolved etiologic questions, the present work again shows that HA and LA do not only differ behaviorally, but also at neurochemical level.

4.7. The role of dispositions

The present work shows that several outcomes of multiple MDMA treatments were partly dependent on subject-inherent factors. These differential effects encompassed all levels of analysis, namely psychomotor activation, behavior in the EPM and novel object test, body weight, and neurochemistry. Overall, physiological effects (neurochemistry, body weight loss) were more conspicuous in case of HA rats, whereas the behavioral effects of multiple MDMA treatments occurred especially in LA rats. Interestingly, such LA rats had also been more vulnerable to lasting behavioral consequences of a single dose of MDMA (7.5 mg/kg [23]).

In trying to characterize the patterns of changes in LA rats, one could conclude that they were somehow behaviorally disinhibited by multiple MDMA treatments, since they showed more locomotion in the activity box, spent more time in the open arms of the elevated plus-maze (min6–10) and responded stronger to the unfamiliar object in the novel object test. The physiological basis of these MDMA effects remains unclear, but the present data seem to indicate that the behavioral effects and those on biogenic amines are dissociated, since the former effects were more prominent in case of LA rats, whereas neurochemical ones were detected in HA rats. It should be reminded, however, that 5HT and DA are not the only neuromodulators, which mediate the outcomes of MDMA or which affected by it. Cytokines, for example, could also be critical: It has been postulated that MDMA acts as a chemical stressor on the immune system [90]. Among others, the drug increases the immunosuppressive cytokine interleukin-2 [91], and interestingly, interleukin-2 mRNA levels differ between HA and LA rats in striatum and frontal cortex [21,22]. Thus, in future work, possible MDMA effects on brain cytokines should also be investigated in HA and LA rats.

Together, the present findings provide further experimental evidence in support of the assumption that between-subject

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variability of EPM performance, i.e. anxiety-related behavior, reflects the expression of a disposition or a trait-like factor [18], which can determine the outcome of certain pharmacological treatments. Thus, not only strain, or rat line, has a substantial influence [16], but also the subject within a given line, at least in the case of male Wistar rats. Here, an individual disposition seems to be of specific importance since it can modulate the consequences of a single [23] and repeated MDMA treatment (present results). Importantly, our data do not show that a specific sub-group is generally more vulnerable to the effects of MDMA, but that the outcomes reflect interactions between subgroup, drug, and type of dependent measure. In so far, our data seem to model the clinical situation rather well, where the occurrence of MDMA deficits is known to depend on interactions between several factors, including intake patterns, time after intake, task demands, and personality. Experimentally applying such subject-dependent differential approaches may therefore serve as a preclinical model, since personality traits have been discussed to affect MDMA consumption and its consequences in humans [92]. Thus, individual factors should receive more attention in future research both, pre-clinically and clinically.

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